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EFFECTS ON MEN AT REST OF BREATHING OXYGEN-RICH GAS MIXTURES.

By FRANCIS G. BENEDICT and HAROLD L. HIGGINS.

[*Contribution from the Nutrition Laboratory of the Carnegie Institution of Washington, Boston, Massachusetts.*]

IN studies on the respiratory exchange of man, it has been commonly assumed that the metabolism is essentially the same irrespective of whether pure atmospheric air or air of varying composition is breathed. With the Pettenkofer-Voit respiration apparatus,[1] with the Jaquet respiration chamber,[2] with the apparatus of Speck,[3] and with the Zuntz-Geppert[4] apparatus, normal atmospheric air is usually inspired, but in the development of the respiration apparatus[5] at Wesleyan University on the principle of Regnault-Reiset,[6] it was soon seen that appreciable differences might exist in the composition of the air inside the chamber, *i. e.*, the air inspired by the subject.

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[1] PETTENKOFER: Annalen der Chemie und Pharmacie, 1862, Supp. Bd. ii, pp. 1–52.

[2] JAQUET: Verhandlungen der naturforschenden Gesellschaft in Basel, 1904, xv, p. 252.

[3] SPECK: Physiologie des menschlichen Athmens, Leipsic, 1892, p. 7; Schriften der Gesellschaft zur Beförderung der gesammte Naturwissenschaften zu Marburg, 1871, x.

[4] See detailed description of the Zuntz-Geppert apparatus by MAGNUS-LEVY: Archiv für die gesammte Physiologie, 1895, lv, p. 1.

[5] ATWATER and BENEDICT: Carnegie Institution of Washington Publication No. 42, 1905.

[6] REGNAULT and REISET: Annales de chimie et de physique, 1849, ser. 3, xxvi, p. 299.

With the establishment of the Nutrition Laboratory, a new respiration apparatus [7] for use with man has been developed in which the closed system of ventilation is also employed; and as the volume of air in the system is only about 15 litres, there may be a considerable fluctuation in the oxygen-content of the inspired air. The construction of the apparatus is such that the air breathed by the subject is invariably carbon-dioxide free, but the oxygen-content may be varied considerably, and it is possible to secure atmospheres of oxygen with any degree of purity up to 90 per cent, or, indeed, above this amount, at a relatively slight expense. With the development of this apparatus, therefore, and its introduction into the regular routine work of the laboratory, it became apparent that an extensive study of the influence of the respiration of oxygen-rich mixtures was possible and much needed. Furthermore, without taking into consideration the use of oxygen in therapy, the results of such a study would have considerable practical value in view of the increasing use of oxygen-rich atmospheres in connection with diving dresses, submarine life-saving appliances, and rescue apparatus for mines.

Earlier Investigations.

As early as the latter part of the eighteenth century, Lavoisier and Seguin [8] made experiments which led them to the conclusion that there was no increase in the vital processes as the result of breathing pure oxygen. These observations of Lavoisier were substantiated sixty years later by Regnault and Reiset [9] in experiments on animals. That the question was not satisfactorily settled in the minds of all scientists, however, is evidenced by the fact that Paul Bert [10] made a number of experiments with oxygen-rich atmospheres, using oxygen under a high pressure. As a result of these experiments, he asserted that with 40 to 50 per cent of oxygen there existed a certain optimum pressure at which larger amounts of oxygen were absorbed than either below or above this point. With very rich oxygen mixtures the metabolism was essentially that of normal air, while with a content of 40 to 50 per cent of oxygen there was a considerable increase in the oxidation.

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[7] Benedict: This journal, 1909, xxiv, p. 345.

[8] Lavoisier and Seguin: Mémoires de l'Académie des Sciences, 1789, p. 185.

[9] Regnault and Reiset: *Loc. cit.*

[10] Paul Bert: La pression barométrique, Paris, 1878, p. 654.

Later Speck,[11] with his improved methods for studying the respiratory exchange of man, began a series of investigations in which relatively rich oxygen mixtures were inspired. Using the spirometer method, he came to the conclusion that the oxidative processes were not affected in any way by the increased oxygen-content of the inspired air.

An early practical use of atmospheres rich in oxygen was made in 1879 by Mr. Fleuss, master-diver of Siebe, Gorman & Co. of London. Using a diving helmet in which oxygen was supplied directly and without the usual diving pump, Mr. Fleuss spent an hour or more under water in a large diving tank.[12] Unfortunately no gas analyses were made, but it is highly probable that for this period Mr. Fleuss was breathing an atmosphere containing not far from 50 to 60 per cent of oxygen.

Lukjanow [13] in 1884 published a series of experiments with a modified form of a Regnault-Reiset apparatus in which he used animals and birds. The oxygen-content of the air inspired varied from 20 to 30 per cent and from 80 to 90 per cent. His results show that there was a very great variation in the oxygen consumed with the oxygen-rich air.

Tobieson,[14] making experiments with a dog, found results which indicated that the oxygen consumption during the inspiration of oxygen-rich air may vary. He also concluded that any increase in the oxygen consumption was shown by his experiments to be dependent upon the nervous system.

Loewy,[15] following the specific directions of Speck, found that the actual consumption of oxygen was unaltered when the inspired air contained as much as 45 per cent of oxygen.

[11] Speck: Archiv für die gesammte Physiologie, 1879, xix, pp. 171–190.

[12] B. W. Richardson: Nature, 1879, xxi, p. 62.

[13] Lukjanow: Zeitschrift für physiologische Chemie, 1884, viii, p. 324.

[14] Tobieson: Skandinavisches Archiv für Physiologie, 1895, vi, p. 292.

[15] Loewy: Untersuchungen über die Respiration und Cirkulation bei Aenderung des Druckes und des Sauerstoffgehaltes der Luft. Berlin, 1895. Abth. II, p. 59, and Abth. V, p. 139. Cited by Magnus-Levy: Physiologie des Stoffwechsels, p. 273.

In 1902 a series of experiments on animals made by Rosenthal[16] indicated an enormous absorption of oxygen by the animals he employed, following a relatively small increase in the percentage of oxygen in the air. This brought up the question as to the storage of oxygen in the body, and Rosenthal maintained that very considerable quantities of oxygen can be thus stored.

Working independently and with different kinds of apparatus, Durig[17] in Vienna and Schaternikoff[18] in Moscow, carried out extensive series of investigations with men and dogs to test Rosenthal's theory. Durig's experiments were made with his usual careful technique on an apparatus in which some of the Zuntz gas analysis apparatus played an important rôle. The published report of his experiments is extremely critical and complete, and is accompanied by an excellent review of all the literature up to that date. Using a modified form of closed-circuit apparatus in which the carbon dioxide was absorbed by caustic potash, Durig found that there was neither an increased consumption nor storage of oxygen by men or dogs on breathing an oxygen-rich mixture. Working with an entirely different form of apparatus, Schaternikoff found with a man exactly the same results reported by Durig. Thus, both of these writers with modern technique have simultaneously refuted the statements of Rosenthal.

The marked effect of an oxygen-rich mixture on the mechanics of respiration has been noted in a number of cases by Hill and Flack.[19] Not only these authors,[20] but also Haldane and Priestley,[21] mention that in holding the breath oxygen-lack is a more potent factor than carbon dioxide and much more stimulating to the respiratory centre, a view substantiated by Leimdörfer[22] and by Hough,[23] who showed recently that high oxygen delays hyperpnœa on breathing in and out of a closed system.

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[16] ROSENTHAL: Archiv für Physiologie, 1902, Supp. Bd., p. 293.

[17] DURIG: Archiv für Physiologie, 1903, Supp. Bd. pp. 209–369.

[18] SCHATERNIKOFF: Archiv für Physiologie, 1904, Supp. Bd. pp. 135–166.

[19] HILL and FLACK: British medical journal, 1908, August 22, p. 499, and October 3, p. 967; *ibid.*, 1909, November 27, p. 1522; Journal of physiology, 1909, xxxviii, Proceedings, p. i.

[20] HILL and FLACK: Journal of physiology, 1908, xxxvii, p. 77.

[21] HALDANE and PRIESTLEY: Journal of physiology, 1905, xxxii, p. 225.

[22] LEIMDÖRFER: Biochemisches Zeitschrift, 1909, xxii, p. 45.

[23] HOUGH: This journal, 1910, xxvi, p. 156.

A critical examination of the literature shows us that practically all of the results of the experiments made on the effect of oxygen-rich mixtures, with the subject at rest, are in opposition to the theory of Rosenthal and strongly favor the belief expressed by Lavoisier that increased oxygen in the air does not materially affect the metabolism. Nevertheless we find that there is much that is lacking in the evidence thus far secured, so that the matter is not yet definitely settled.

DEFECTS IN THE EARLIER INVESTIGATIONS.

In the earlier investigations but relatively few experiments have been made, since each investigator was content with a small number of experiments. Secondly, very few investigators used more than one subject; and thirdly, many of the experiments were made upon animals and not on men.

Objections can furthermore be made to the earlier work in that the experiments were not always carried out under normal conditions of rest. For instance, in the otherwise well-planned research of Durig, a maximum expiration both began and ended the experiment. This is objectionable, as the maximum expiration involves considerable muscular work in excess of that in normal breathing; moreover, with a maximum expiration, there is a disturbance in the composition of the alveolar air at the very start of the experiment. As a consequence, the experimental conditions cannot be termed strictly those of "normal rest." A factor that is probably not without significance is the mental state of "attention" required of the subject by this use of the maximum expiration, owing to his watching for the signal. In much of the earlier work, likewise, the subject was called upon more or less to assist personally in the experiments by manipulating valves, operating stopcocks, etc. The muscular work involved therein has been pointed out by Durig as actually being of disadvantage in an experiment. Furthermore, in comparing the respiratory exchange for ordinary air with the respiratory exchange for high oxygen, the observations used in most instances were not made on the same day, and frequently were separated by several days.

Throughout all of the investigations we also fail to find adequate records of the effect of the inhalation of high oxygen on other factors besides the oxygen consumption, such as the pulse and respiration rates, and the carbon dioxide excreted. To be sure, in certain experiments the ventilation of the lungs was observed, which is an important factor, but there is little evidence to show that there may not have been a change in character of the metabolism, and there is no evidence bearing on the possibility of there being a different sort of body material burned in the presence of high percentages of oxygen.

A further criticism of previous investigations may be made in that the experiments have not been sufficiently numerous and well grouped to throw light upon any slight differences in the oxygen absorption as might become apparent if a large number of experiments were averaged.

Finally, all the methods of studying the respiratory exchange of men heretofore employed have involved the use of elaborate gas analysis apparatus. Differences in gas analyses at the beginning or end of an experiment which might appear to be minute may easily play an important rôle even to the extent of completely masking (or implying) an actual consumption of oxygen. This is clearly shown by Durig in his thorough criticism of Rosenthal's investigations.

PURPOSE AND SCOPE OF THIS INVESTIGATION.

In the investigation here reported, an effort was made to determine the influence, if any, of the inspiration of oxygen-rich mixtures upon the metabolism and the mechanics of respiration. To this end short metabolism experiments were made, lasting from ten to thirty minutes, in which the respiration apparatus previously mentioned was used. In these experiments the subject breathed either ordinary air or a gas mixture containing 40, 60, or 90 per cent of oxygen by volume.

In order to obtain a true comparison of the metabolism, a series of experiments with normal air was made on every day that experiments were made with an atmosphere containing a high percentage of oxygen. If on one day the experiments with a high percentage of oxygen preceded the series with normal air, on a subsequent day the order would be reversed. In fact, the experimental conditions were carefully arranged so that the time of day, duration of an experiment, degree of activity, and previous diet would be the same in experiments with normal air as in those with 40, 60, or 90 per cent of oxygen with which they are compared. The experiments were all made with men, at least twelve hours after the last meal, and with the subjects lying on a couch at complete muscular rest. The experiments also allowed us to note any possible effect of changing from a normal atmosphere to one containing a higher percentage of oxygen and *vice versa*.

In addition to studying the oxygen consumption of the subjects, we have studied the metabolism as indicated by the carbon dioxide output and the respiratory quotient. Furthermore, data were collected regarding the mechanism of respiration and circulation as indicated by the pulse and respiration rates and by the graphic records of the breathing as shown on a kymograph connected with a pneumograph placed about the chest of the subject.

Finally, the investigation was so planned as to include a large number of experiments with several subjects, and a considerable amount of data has thus been secured from which to draw the final deductions. Every effort was made to eliminate the personal equation and any psychical effects, and all the errors incidental to earlier researches were avoided so far as possible. This investigation did not include in any way experiments with gas mixtures containing a percentage of oxygen materially lower than that of normal air, nor of carbon dioxide mixtures, the inspired air being invariably carbon-dioxide free.

Apparatus used in the Research.

With the apparatus used in this investigation, the subject is not enclosed in a chamber, but may lie on a comfortable couch, sit in a chair, stand, or do muscular work. A detailed description of the apparatus has been given in a previous paper,[24] which includes also a statement of the fundamental principles involved in its construction, and the method of use.

Since the completion of the investigation several new features have been introduced in the apparatus which have further increased its efficiency. A pneumatic method of throwing the valve which opens or closes the ventilating system to the outside air has been substituted for the method previously used. Changes have also been made in the forms of some of the absorbers. In the earlier development of the apparatus a silver-plated brass can, closed with a rubber gasket and collar, was used for the carbon dioxide absorber. For this has been substituted a wide-mouth chemical bottle, with a capacity of about 2000 c.c. This is closed with a rubber stopper, into which are fitted two wide glass or metal tubes; one of these tubes extends nearly to the bottom of the bottle, while the other reaches only to the bottom of the stopper.

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[24] Benedict: This journal, 1909, xxiv, p. 345.

A new form of water absorber, recently presented to the Nutrition Laboratory by Professor Graham Lusk, has also been substituted for the sulphuric acid container in which the water from the soda lime is absorbed. This vessel was designed by Dr. H. J. Williams of the Department of Physiology of the Cornell University Medical School. It is most ingeniously and skillfully made of blown glass,[25] and the preliminary tests with it have been so uniformly successful and it has proved so efficient that we have made it a permanent part of the apparatus.

ROUTINE OF EXPERIMENTS.

The general routine of experiments with the respiration apparatus is outlined in the paper previously referred to,[26] but there were many features of the experiments with a high-oxygen atmosphere which merit special attention. A definite routine was followed which varied but little throughout the research. All of the experiments were made in the morning, beginning about eight o'clock, and the subject came to the laboratory without breakfast. By thus making the experiments at least twelve hours after the last meal, it was the intention to avoid the effect of any work involved in the digestion of food previously taken, and to avoid particularly the specific dynamic action of the protein. Benedict, Emmes, and Riche [27] have recently shown that there is a considerable difference in the character of the metabolism twelve hours after the last meal, this difference depending upon whether the food eaten was rich or poor in carbohydrates. Nevertheless, the total oxygen consumption does not vary materially at this time unless the previous meal has contained a large amount of protein.

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[25] E. MACHLETT & SON of New York City.

[26] BENEDICT: *Loc. cit.*, p. 365.

[27] BENEDICT, EMMES, and RICHE: This journal, 1911, xxvii, p. 383.

The subject, soon after his arrival at the laboratory, lay down on a couch and remained in this position until the experimenting was over. In order to avoid any influence of previous activity on the metabolism, experimenting was not begun until the subject had been quietly lying on the couch twenty or thirty minutes. During the experimental periods themselves the subject also remained as quiet as possible, and usually made no major muscular movements, except, perhaps, to change the position of the hands. Between the experiments the nosepieces were deflated and the subject changed his position, if he desired, although further muscular activity was avoided. The first experimental period was usually ten minutes in length and subsequent ones fifteen minutes. Experiments on several occasions were continued twenty or thirty minutes, for the purpose of noting any changes in metabolism which might result from breathing oxygen-rich air for over fifteen minutes.

The experiments with a high percentage of oxygen were made in exactly the same manner as those with normal air, special precautions being taken in weighing. Atmospheres of the desired tension were obtained by means of a simple technique which involved the repeated filling with oxygen of the tension equalizer[28] on the apparatus and the thorough mixing of the air after each filling. In obtaining atmospheres containing 90 per cent of oxygen, a second pan and diaphragm were used in addition to the tension equalizer. After an experiment with an atmosphere containing a high percentage of oxygen, dry air of normal composition, free from carbon dioxide, was passed through the carbon dioxide absorber and its adjacent water absorber before they were weighed. This insured their being weighed with a content of normal air as in the preliminary weighing, and not with a content of the heavier oxygen-rich air.

The work of an experimental day consisted first of three experiments in which the subject breathed normal air; if the agreement of the results in these three experiments was not close, a fourth experiment was made with the same atmosphere. Then three experiments would be made with an oxygen-rich atmosphere containing 40, 60, or 90 per cent of oxygen. Experiments were made with only one of these oxygen-rich atmospheres on any day. After the conclusion of these experiments, frequently a final experiment was made with normal air. On a subsequent morning the same routine would be followed except that the order was reversed and the experiments with an oxygen-rich atmosphere were made first. By averaging the results of the work of these two mornings, it would seem that fair averages might be obtained for comparing the results of breathing an atmosphere with a high percentage of oxygen with the results obtained with normal air. Checks on the calculated amounts of oxygen present in the system were made by the Haldane gas analysis apparatus.[29]

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[28] BENEDICT: *Loc. cit.*, p. 357.

STATISTICS OF EXPERIMENTS.

In the course of this investigation six different subjects were used, and 292 experiments were made with satisfactory results. Of these, 156 experiments were made with ordinary air, 49 experiments with 40 per cent of oxygen, 32 experiments with 60 per cent of oxygen, and 55 experiments with 90 per cent of oxygen. The subjects were all young men in good health and connected with various investigations in the laboratory. The physical data with regard to height and naked body weight are given in Table I.

TABLE I.

STATISTICS OF HEIGHT AND AVERAGE WEIGHT OF SUBJECTS.

Subject.	Height.	Body weight without clothing.
	cm.	kilos.
J. J. C.	175	66
L. E. E.	179	60
H. L. H.	172	60
D. J. M.	175	58
A. G. E.	169	57
T. M. C.	166	49

Having described the method by which the experiments were made, it is not considered advisable to give in detail the values for the individual experiments with each subject. As the worth of a method, however, frequently depends upon the ability with which constant results can be obtained, we present in Table II the results of all of the experiments made with one subject, numbered in serial order for each day of experimenting. The values for T. M. C. given in this table are typical of the results obtained with the other subjects.

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[29] HALDANE: Journal of physiology, 1898, xxii, p. 465.

The subject T. M. C. had had a long experience with experiments on the respiratory exchange, understood the significance of the experiments themselves, and co-operated in every way towards their success. The respirations were most regular, and the subject was not in any way affected by extraneous movements in the laboratory. Throughout the experiments the body weight remained practically constant, the periods for comparison being rarely more than a few minutes apart; accordingly it was not considered necessary to reduce all values to the basis of per kilogram of body weight.

The striking feature of Table II, irrespective of the percentages of oxygen in the inspired air, is the remarkable constancy of the amount of carbon dioxide produced and of oxygen absorbed per minute. If we consider the whole series of experiments extending from February 7 to June 20, and omit the obviously abnormal value of 181 in the first experiment on March 26, we find that the minimum carbon dioxide excretion was 148 c.c. per minute, and the maximum 168 c.c. per minute. It is thus seen that the carbon dioxide produced was remarkably constant. The oxygen absorption varied from a minimum of 173 c.c. per minute to a maximum of 209 c.c. per minute, variations no larger than would be expected with a series of experiments extending over so long a period, with perhaps different planes of nutrition and different atmospheric conditions.

The high carbon dioxide value and the high respiratory quotient of 1.00 in the first experiment on March 26 can be readily explained by the abnormally high carbon dioxide exhalation. It is seen that the oxygen consumption in the series of experiments on this day was relatively constant at 180 c.c., while the carbon dioxide excretion is very much higher in the first experiment than in the others. This may be due to an inequality of muscular activity, as the first experiment of a series frequently tends to be somewhat abnormal. For that reason it is usually only ten minutes long, and in a few instances can be considered only as a preliminary observation. A close examination

TABLE II.

Respiratory Exchange of Subject T. M. C. while breathing Gaseous Mixtures with Varying Oxygen Tensions.

Date.	Expt. No.	Duration.	Percentage of oxygen.	Pulse rate per minute.	Respiration rate per minute.	Carbon dioxide produced per minute.	Oxygen absorbed per minute.	Respiratory quotient.
1910		min.				c.c.	c.c.	
Feb. 7	1	15	20	74	14	158	193	0.82
	2	16	20	73	14	157	184	0.85
	3	15	20	74	15	165	193	0.85
	4	15	40	75	14	163	195	0.84
	5	15	40	74	15	164	203	0.81
	6	15	40	74	14	168	209	0.80
Feb. 23	1	10	20	71	15	152	191	0.80
	2	15	20	71	14	155	181	0.86
	3	15	20	71	15	150	180	0.83
	4	15	20	68	15	152	180	0.84
	5	15	40	69	15	148	182	0.81
	6	15	40	67	16	150	186	0.81
	7	15	40	70	15	156	188	0.83
March 23	1	10	40	68	16	164	196	0.84
	2	15	40	66	14	152	188	0.81
	3	16	40	67	14	152	189	0.80
	4	15	20	67	13	157	192	0.82
	5	15	20	68	13	159	184	0.86
	6	15	20	67	14	150	...	...
	7	15	20	68	16	152	178	0.85
March 26	1	10	40	72	14	181	181	1.00
	2	15	40	72	15	164	179	0.92
	3	15	40	67	14	158	175	0.90
	4	15	40	67	15	161	181	0.89
	5	16	20	69	14	158	182	0.87
	6	16	20	69	14	157	179	0.88
	7	16	20	69	15	161	186	0.87
May 25	1	10	20	70	14	162	173	0.94
	2	15	20	70	13	155	179	0.87
	3	15	90	63	12	151	174	0.87
	4	15	90	66	13	154	183	0.84
	5	15	90	65	14	152	179	0.85
June 2	1	16	20	70	14	150	...	...
	2	17	20	68	14	152	174	0.87
	3	15	90	64	15	153	176	0.87
June 8	1	12	90	71	14	161	190	0.85
	2	15	90	71	14	155	191	0.81
	3	15	90	69	15	162	183	0.89
	4	15	20	71	13	156	190	0.82
	5	15	20	73	15	152	186	0.82
	6	15	20	72	13	157	186	0.84
	7	15	90	69	12	162	187	0.87
June 20	1	13	90	76	13	155	185	0.84
	2	15	90	75	13	...	181	...
	3	16	90	71	14	154	...	...
	4	15	20	74	14	151	188	0.80
	5	15	20	75	13	150	187	0.80
	6	15	90	68	13	154	182	0.85

of any series of three experiments, made under like conditions, shows that the results are very constant, and that, aside from the single abnormal instance cited above, they can be looked upon as true duplicate experiments.

DISCUSSION OF RESULTS.

In the conduct of this research the percentage of oxygen was increased gradually with nearly all of the subjects. Thus, with any particular subject, experiments were first made with an atmosphere containing 40 per cent of oxygen, and subsequently the percentage of oxygen was increased to 60 and 90 per cent respectively. Since there was no material difference in the results of the experiments with the various percentages of oxygen, we have selected for special discussion the most extended series, *i. e.*, those with an atmosphere containing 90 per cent of oxygen. These results, together with the results of the series of experiments with an atmosphere containing 20 per cent of oxygen, are given in Table III.

COMPARISON OF THE RESULTS OBTAINED WITH 20 PER CENT AND 90 PER CENT OF OXYGEN.

The results obtained in these two series of experiments may be presented in a variety of ways and a number of different averages drawn. After a careful consideration of the points involved, we have selected the following method of presentation and averaging.

As has previously been stated, on every experimental day two or three series of experiments were made; the daily averages for these different series have been grouped together chronologically for the respective subjects in Table III. As the experiment in Series III was only a supplementary test and not made on every day, it was not averaged with the experiments in Series I, notwithstanding the fact that it was made with the same percentage of oxygen as those in Series I.

A general average for each subject is also given in Table III. In an attempt to eliminate the possible influence of preceding muscular activity, time of day, and adjustment to the apparatus, we have calculated this average in the following manner. When the number

TABLE III.

COMPARISON OF EXPERIMENTS WITH 20 PER CENT AND 90 PER CENT OF OXYGEN.

Subject.	Date.	Series No.	Percentage of oxygen.	Pulse rate per minute.	Respiration rate per minute.	Carbon dioxide produced per minute.	Oxygen absorbed per minute.	Respiratory quotient.	Number of experiments averaged and their duration.
	1910					c.c.	c.c.		
T. M. C.	May 25	I	20	70	13.5	159	176	0.90	1–10 min., 1–15 min.
		II	90	65	13	152	179	0.85	3–15 min.
	June 2	I	20	68	14	152	174	0.87	1–15 min.
		II	90	64	15	153	176	0.87	1–15 min.
	June 8	I	90	70	14.5	159	188	0.85	1–12 min., 2–15 min.
		II	20	72	13.5	155	187	0.83	3–15 min.
		III	90	69	12	162	187	0.87	1–15 min.
	June 20	I	90	76	13	155	185	0.84	1–12 min.
		II	20	75	13.5	151	188	0.80	2–15 min.
		III	90	68	13	154	182	0.85	1–15 min.
	Average .	. . .	**20**	**71**	**13.5**	**154**	**181**	**0.85**	
			90	**69**	**14**	**155**	**182**	**0.85**	
J. J. C.	April 4	I	20	61	17	187	224	0.83	1–15 min., 1–30 min.
		II	90	54	16.5	181	209	0.87	2–30 min.
		III	20	61	19	188	234	0.80	1–15 min.
	April 7	I	20	67	16.5	193	228	0.85	1–10 min., 1–30 min.
		II	90	56	16	185	219	0.84	2–30 min.
		III	20	60	16	184	214	0.86	1–15 min.
	May 6	I	20	58	16.5	184	222	0.83	1–12 min., 2–15 min.
		II	90	54	17	181	214	0.85	1–15 min.
	June 6	I	20	63	17	197	232	0.85	1–12 min., 2–15 min.
		II	90	52	16.5	189	224	0.84	3–15 min.
		III	20	56	16	186	208	0.89	1–15 min.
	June 10	I	90	63	18.5	189	230	0.82	1–12 min., 1–15 min.
		II	20	59	16	185	222	0.83	2–15 min.
		III	90	51	16	177	221	0.80	1–15 min.
	Average .	. . .	**20**	**61**	**16.5**	**188**	**225**	**0.84**	
			90	**59**	**17.5**	**187**	**224**	**0.83**	

A. G. E.		II	90	61	13.5	199	211	0.94	1–30 min., 1–15 min.
	May 19	I	20	71	12.5	205	219	0.94	2–12 min., 1–15 min.
		II	90	63	13	207	223	0.93	1–15 min.
	May 27	I	20	67	12	199	218	0.91	1–10 min., 2–15 min.
		II	90	61	13	199	211	0.94	3–15 min.
	May 31	I	90	62	12.5	200	217	0.92	1–12 min., 2–15 min.
		II	20	70	11.5	196	214	0.92	2–15 min.
	June 3	I	90	60	12	199	213	0.93	1–12 min., 2–15 min.
		II	20	69	11.5	193	221	0.87	2–15 min.
	Average . .	. . .	**20**	**68**	**12**	**198**	**218**	**0.91**	
			90	**62**	**13**	**201**	**215**	**0.93**	
L. E. E.	June 7	I	20	58	12	193	237	0.81	1–12 min., 2–15 min.
		II	90	50	11.5	196	242	0.81	2–15 min.
	June 11	I	90	52	11	197	238	0.83	2–15 min.
		II	20	54	10.5	200	241	0.83	3–15 min.
	Average . .	. . .	**20**	**56**	**11.5**	**197**	**239**	**0.82**	
			90	**51**	**11.5**	**197**	**240**	**0.82**	
H. L. H.	May 23	I	20	72	14.5	203	233	0.87	1–10 min., 2–15 min.
		II	90	63	15	203	227	0.89	2–15 min.
	May 28	I	20	66	12.5	194	234	0.83	1–10 min., 2–15 min.
		II	90	61	14.5	206	246	0.84	3–15 min.
		III	20	64	13	195	246	0.79	1–15 min.
	June 1	I	90	59	13.5	196	239	0.82	1–12 min., 2–15 min.
		II	20	66	13	192	239	0.80	3–15 min.
		III	90	64	12	209	253	0.83	1–15 min.
	June 4	I	90	58	16.5	195	233	0.84	3–15 min., 1–7 min.
		II	20	61	16	198	245	0.81	2–15 min.
	Average . .	. . .	**20**	**66**	**14**	**197**	**238**	**0.83**	
			90	**60**	**15**	**200**	**236**	**0.85**	
D. J. M.	May 20	I	20	61	15	183	240	0.76	1–12 min., 2–15 min.
		II	90	57	16.5	212	256	0.83	3–15 min.
	Average . .	. . .	**20**	**61**	**15**	**183**	**240**	**0.76**	
			90	**57**	**16.5**	**212**	**256**	**0.83**	
	Average . (6 subjects)	. . .	**20**	**64**	**14**	**186**	**224**	**0.83**	
			90	**60**	**14.5**	**192**	**226**	**0.85**	

of experimental days on which the experiments with 20 per cent of oxygen were first made exactly equalled the number of days on which the experiments with 90 per cent of oxygen were made first, the averages for the respective percentages of oxygen were obtained by averaging the daily averages. If, however, they were not exactly equal, all of the experiments with 20 per cent of oxygen in Series I were first averaged, and then the experiments with 20 per cent of oxygen in Series II. These two results were again averaged, and this last value is given in the table as the general average for the subject for this percentage of oxygen. The same method of averaging was followed for the experiments with 90 per cent of oxygen. Finally, the grand average is given for the whole group of subjects for both 20 per cent of oxygen and for 90 per cent of oxygen.

An examination of the figures in this table shows that with subject T. M. C. there was an apparent increase in the oxygen consumption when he was breathing an atmosphere with 90 per cent of oxygen on the first experimental day, and a very slight increase on the second experimental day. On the third day there was a decrease in the oxygen consumption at the lower tension, and a slight increase at the lower tension on the fourth day, followed by one experiment with high tension in which the oxygen consumption fell slightly. The experiments with this subject as a whole show that the oxygen consumption was independent of the oxygen tension in the air inspired. There is, furthermore, no evidence that the order of the experiments influenced the results in any way.

With the subject J. J. C., on April 4, there was a noticeable decrease in the oxygen consumption when changing from 20 per cent to 90 per cent; singularly enough, this subject shows a lower oxygen absorption at the higher tension in all experiments save that of June 10.[30] The average results of the five days with this subject show no difference resulting from either the change in tension of the oxygen or the order in which the experiments were made.

The averages of the results with the subjects A. G. E., L. E. E., and H. L. H. show a strikingly uniform oxygen consumption irrespective of tension. The greatest difference observed in any case where several experiments were averaged was with the subject D. J. M., where the average for three experiments showed 240 c.c. of oxygen absorbed with 20 per cent of oxygen, and 256 c.c. as the average of three experiments with 90 per cent of oxygen. Unfortunately other experiments were not made with this subject. D. J. M. was a very restless subject, and the results of experiments with him are of doubtful value, especially as the change may have been due to muscular activity.

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[30] This comparison applies only to Series I and II, as the one experiment in Series III was a supplementary experiment and not strictly comparable with those in Series I.

The average of the results with all of the six subjects shows almost uniform oxygen consumption under both conditions. The carbon dioxide excretion, respiratory quotient, and respiration rate appear to be unaffected by a change in the oxygen tension, while the pulse rate shows a decrease.

CONCLUSIONS WITH REGARD TO THE OXYGEN ABSORPTION WHEN BREATHING OXYGEN-RICH ATMOSPHERES.

Although in the strict comparison of the oxygen absorption with oxygen tensions varying between 20 and 90 per cent, particular care was taken to have as far as possible an equal number of experiments averaged, it may be seen, from an examination of the daily averages in Table III, that there is practically no difference in the oxygen absorption resulting from the order in which the experiments were made. Inasmuch as we have found this to be likewise true in comparing the results obtained with 40 and 60 per cent of oxygen, we believe that we can make a broad generalization from all of the experiments on the basis of the percentage of oxygen used without particular reference to the actual number of experiments averaged or the order in which they were made. By so doing, it is true that we employ in averaging a much larger number of experiments for 20 per cent of oxygen than for other percentages, but the large number of experiments made with each percentage of oxygen and the remarkable regularity found in practically all of the experiments lead us to believe that this method of averaging and presenting the figures cannot be seriously misleading. This comparison of the results obtained with the different percentages of oxygen is given in Table IV.

An inspection of Table IV shows that there is no apparent variation in the oxygen consumption resulting from the breathing of 20, 40, 60, or 90 per cent of oxygen. It is of interest to note, although the fact has probably no great significance, that in this table, at least, the largest amounts of oxygen consumed were frequently found with an oxygen tension of 40 per cent. This was the case with T. M. C., A. G. E., and L. E. E. One might assume, therefore, that these data in part substantiate the view held by Paul Bert, that with an atmosphere containing 40 to 50 per cent of oxygen there is a larger oxygen consumption. Inasmuch, however, as the amounts are but very slightly above those obtained with 90 per cent of oxygen, the difference is hardly of significance. The maximum difference as compared with the results with 20 per cent of oxygen is that observed with the subject L. E. E., *i. e.*, 7 c.c., or about 3 per cent.

TABLE IV.

AVERAGE ABSORPTION OF OXYGEN IN EXPERIMENTS WITH VARYING OXYGEN TENSIONS. (C.C. PER MINUTE).

Subject.	Twenty per cent of oxygen.	Forty per cent of oxygen.	Sixty per cent of oxygen.	Ninety per cent of oxygen.
T. M. C.	184	189	...	183
J. J. C.	228	226	229	220
A. G. E.	219	221	215	214
L. E. E.	244	251	...	241
H. L. H.	234	...	233	238
D. J. M.	234	235	222	256
Average of all subjects	224	224	225	225

The average results of all the experiments with the six subjects are likewise given in the table. These averages might also be criticised, since the value for 20 per cent of oxygen is found from the averages of experiments with a greater number of subjects than those for 40 and 60 per cent of oxygen, but this general averaging is hardly affected by the seeming inconsistency.

The slight mathematical differences in the oxygen consumption at different oxygen tensions may also be expressed on a percentage basis. To make the comparisons as strict as possible, we have given in Table V only such experiments as are comparable, and equal numbers of experiments with each percentage are averaged. In this table, we find that the slight apparent advantage in the experiments with 40 per cent of oxygen, shown in Table IV, disappears in the data given for A. G. E., but remains in the cases of T. M. C. and L. E. E. While experiments with 40 per cent of oxygen were not made with H. L. H., and none with 60 per cent of oxygen were made with T. M. C. and L. E. E., the values are so nearly uniform that the averages have been given in Table V, although as strict averages they are of course open to objection. From these final averages it can be seen that it apparently made no difference in the results whether the subject breathed ordinary air or air with an oxygen-content even as high as 90 per cent.

TABLE V.

PERCENTAGE COMPARISON OF OXYGEN ABSORBED IN EXPERIMENTS WITH DIFFERENT TENSIONS.

Subject.	Twenty per cent of oxygen.	Forty per cent of oxygen.	Sixty per cent of oxygen.	Ninety per cent of oxygen.
	per cent	per cent	per cent	per cent
T. M. C.	100	102.0	....	100.5
J. J. C.	100	98.0	100.0	99.5
A. G. E.	100	99.5	100.5	98.5
L. E. E.	100	103.0	....	100.5
H. L. H.	100	....	100.5	99.0
D. J. M.	100	100.0	98.0	106.5
Average of all subjects	100	100.5	99.8	100.8

As a rule, owing to the length of time required to replace the air in the system with a high-oxygen atmosphere, the subject usually knew when the high-oxygen atmosphere was used. Notwithstanding the psychical influence of such knowledge, no difference was usually noticed by the subjects between the atmospheres in ease of respiration, odor, comfort, or discomfort.

PHYSIOLOGICAL SOURCES OF ERROR.

In all previous investigations on this subject the methods used have required gas analyses involving accurate sampling and delicate manipulation, but this method does not. In other words, the apparatus measures, first, the entire carbon dioxide output, which of itself is important and significant, but more particularly it measures the absolute amount of gas absorbed without recourse to gas sampling, a measurement of the total air inspired and expired, or an analysis of the expired air. Many of the mechanical difficulties met with in the usual routine of a study of the respiratory gases are thus avoided, but there still remain two possible sources of error of a physiological nature that should be more fully considered.

Two questionable assumptions are made in this research: (1) that the volume of gas remaining in the lungs at the end of a normal expiration is the same at the beginning and end of each experiment; and (2) that the total volume of gas dissolved in the body fluids does not undergo material change during the course of an experiment. The probable error in these assumptions warrants further discussion.

Volume of air in the lungs. — The assumption of constancy in the volume of air in the lungs at the beginning and end of each experiment, affecting as it does all experiments made with this type of apparatus, has been given the most careful consideration. Personal conversation with a large number of physiologists has confirmed us in the belief that under normal conditions, with the subject lying quietly and breathing normally, this assumption is unquestionably correct. This has been further substantiated in two ways:

First, by means of the large plethysmograph of Haldane[31] and also by an Ellis tube pneumograph as used in this laboratory, it seems to be clearly shown that the volume of air in the thorax at the end of a normal respiration remains essentially the same, as the excursions of a pointer resting on a tambour in connection with the pneumograph indicate that the position in which the chest comes to rest after an expiration is always the same. This is strikingly shown in the curve given on p. 24, in which the upper portion represents the end of the expiration.

[31] HALDANE and PRIESTLEY: Journal of physiology, 1905, xxxii, p. 225.

Second, if this volume should vary materially, we would expect to find very large variations in the oxygen consumed from experiment to experiment; for if the volume of air in the lungs is greater at the end than at the beginning, the amount expelled into the system would be too small, and pure oxygen must be added from the weighed cylinder to make good the deficiency. On the other hand, if the volume of air in the lungs is less at the end than at the beginning, an excessive amount has been expired into the system, and the air thus added takes the place of oxygen that otherwise would have been admitted from the cylinder.

As a matter of fact, an examination of the data shows that the oxygen consumption, even in individual experiments, is remarkably constant. It seems clear, therefore, that for purposes of comparison we can assume that in these experiments the amount of air in the lungs after a normal expiration remained constant.

Gases dissolved in the body fluids. — While the method of studying the respiratory exchange used in these experiments includes the measurement of the volume of gas absorbed out of a closed system into which the subject breathes, it does not differentiate between oxygen and nitrogen in this gas, but assumes that the gas absorbed out of the system is oxygen, and nothing else. The volume of oxygen required to replenish the gas removed from the system by the respiratory processes of the subject is considered to be a measure of the oxygen consumed.

If, however, in experiments where the tension of oxygen in the gaseous mixture in the system is greater than that of normal air, there has been an interchange between the oxygen in the system and the nitrogen in the lungs, obviously the nitrogen has simply been transferred from the lungs to the ventilating air-current. On the other hand, this loss of nitrogen from the lungs must be replaced by an equal volume of gas, which is obtained from the oxygen-rich mixture inhaled. This interchange between the gaseous nitrogen in the lungs and the gaseous oxygen previously in the system, if a mere substitution, would have no effect upon the measurement of the oxygen actually absorbed. In an experiment with a high percentage of oxygen, therefore, the fact that the lungs at the end of the experiment contain perhaps 85 per cent of oxygen, whereas at the start they contained but 15 per cent, would not affect our calculations of the oxygen consumed inasmuch as the volume is unchanged.

But there is in this connection a possible opportunity for error that must be carefully considered. In the blood plasma and body fluids, definite amounts of oxygen and nitrogen are dissolved under the conditions existing when normal air is breathed. When an atmosphere rich in oxygen is inspired, there is a change in the partial pressure of the oxygen in the alveolar air, and consequently there will be a certain absorption of oxygen accompanied by the giving up of nitrogen. Unless these two compensate, an error may result. The researches and calculations of Durig[32] imply that this compensation is reasonably complete. Unfortunately we have at present no special experimental evidence to contribute on this point other than that afforded by a comparison of several experiments which lasted thirty minutes with those which lasted fifteen minutes. Any discrepancy between the absorption of oxygen by the body fluids and fat and the giving up of nitrogen would naturally be more apparent in the shorter experiments, since the longer the period of the experiment the more time there would be for the establishment of an equilibrium. As a matter of fact, the oxygen consumption was apparently unaffected by the length of the experiment.

CARBON DIOXIDE EXCRETION AND RESPIRATORY QUOTIENT.

While, as has been shown, the amount of oxygen absorbed is not apparently affected by the percentage of oxygen in the air breathed, there still remains the possibility of an alteration in the character of the metabolism when the blood plasma is saturated, so to speak, with oxygen. Under these conditions an examination of the carbon dioxide output and the respiratory quotient should show any noticeable alteration, if present, in the character of the metabolism. If, for example, a larger proportion of glycogen or carbohydrate is burned, the respiratory quotient would be materially increased. An examination of Table III, which compares the results with 20 per cent and 90 per cent of oxygen, shows that the carbon dioxide output indicated by the averages for all of the experiments was essentially the same with both, being perhaps slightly lower with the oxygen-rich atmosphere. As no physiological significance can be ascribed to the slight differences of one or two points in the second decimal place, it is apparent that the respiratory quotient was practically unaltered. We must therefore conclude that the inhalation of pure oxygen does not produce any measurable change in the character of the metabolism during the time that the experiment is in progress.

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[32] DURIG: Archiv für Physiologie, Supp. Bd., 1903, p. 209.

MECHANICS OF RESPIRATION AND CIRCULATION.

Respiratory volume and rate. — The volume of air respired and the character of the respiration were observed by means of a pneumograph fastened around the chest. A study of a number of the curves obtained in these experiments shows that the amplitude of the pointer indicates no alteration in the character or size of the respiration when the change was made from one atmosphere to another; we must therefore conclude that the inhalation of a high percentage of oxygen did not produce an alteration in the character or volume of the respiration or in its frequency. This is not in accord with the observations of Schlesinger and Pembrey,[33] as they found an average increase of 80 c.c. in each respiration when the subjects breathed pure oxygen. Three curves obtained with T. M. C. are reproduced in Fig. 1 to illustrate the variation in the respiration during an experiment, and will serve as types of the curves obtained during the different experiments.

These curves were obtained in three experiments in which the subject breathed ordinary air during the preliminary period and then a change was suddenly made to air containing 90 per cent of oxygen. Records were obtained with two pneumographs, and a time record in minutes was also made. As will be seen by the numbering, the records for the first experiment are given at the bottom of the figure, and those for the third experiment at the top. Of the three records for each experiment, the upper (*P*–1) shows the curve for the pneumograph fastened around the chest, the lower (*P*–2) gives that for the pneumograph about the hips,[34] while the one between (*T*) gives the record of the time in minutes. The beginning and end of the experiments are indicated on this record by the marks lettered *S* and *E*

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[33] SCHLESINGER and PEMBREY: Journal of physiology, 1908, xxxvii, Proceedings, p. lxix.

[34] This pneumograph is used primarily to indicate any major body movements other than respiration.

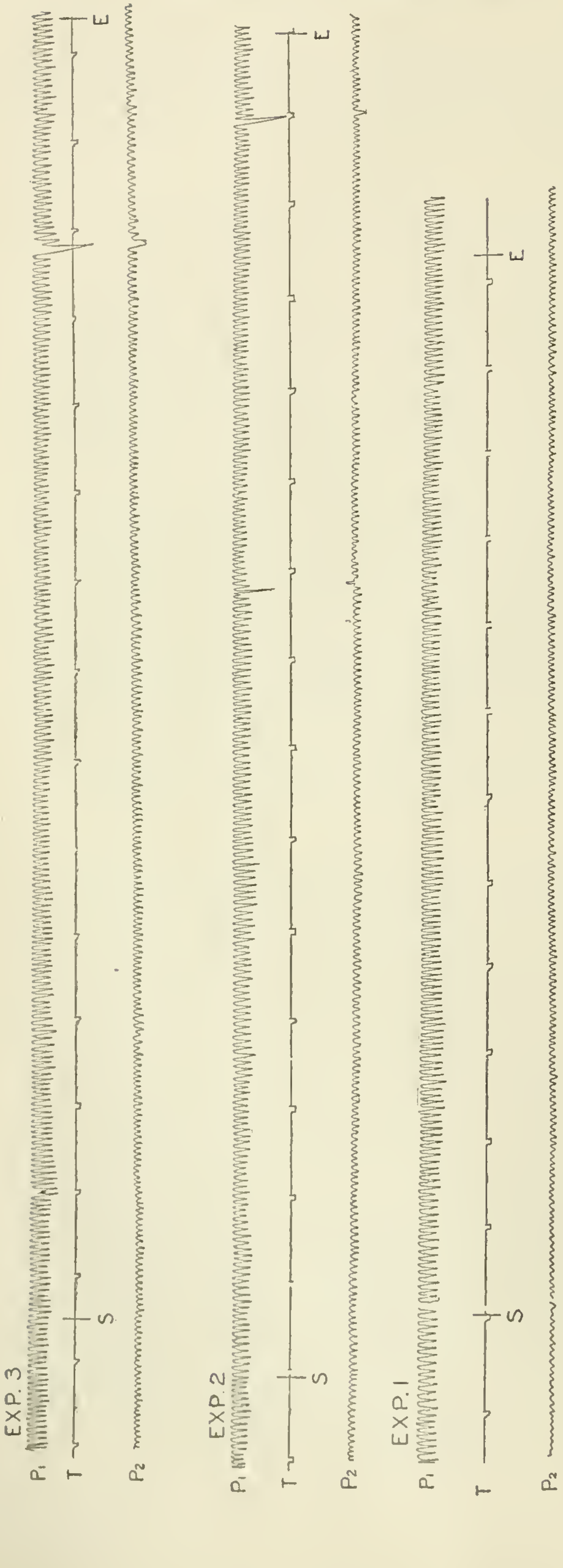

Figure 1. — Pneumograph curves in three experiments with T. M. C. on June 8, 1910, showing the respiration while breathing ordinary air, and air containing 90 per cent of oxygen.

respectively. In Experiments 1 and 2 the beginning is coincident with the time markings; in Experiment 3 the marks are in both instances about half-way between two time marks. The regularity of respiration and the remarkable uniformity at the end of the expiration, namely, the upper part of the curve, is shown in all of the pneumograph curves.

In the curve for Experiment 1 the subject was breathing room air for about one and one-half minutes before the experiment began. When the valve was thrown, no difference was noticeable in the amplitude or rapidity of respiration; likewise after the valve was thrown at the end, there was no change in the rapidity, and but a very slight increase in the amplitude. The record is also given for one minute before Experiment 2 began, and here again there is no noticeable difference in the character of the respiration at the time the valve was thrown. The curve does not show the respiration after the valve was thrown at the end of the experiment. In Experiment 3 the respiration is indicated for one and one-half minutes before the experiment began. The amplitude of the pneumograph pointer is somewhat larger throughout the whole of this experiment, due, possibly, to a slight shifting in the position of the pneumograph. In this experiment, also, no alteration in the character, rapidity, or volume of the respiration on turning the valve can be observed. The curve does not show the results after the valve was thrown at the end of the experiment.

Pulse rate. — An examination of the protocols shows us that while the change from 20 per cent to 40 per cent of oxygen was not accompanied by any material alteration in the pulse rate, there was a slight, though noticeable, decrease when changing from 20 to 60 per cent of oxygen. With 90 per cent of oxygen there was a very positive decrease in the pulse rate, as may be seen from the data recorded in Table III, and this feature of the research seems to warrant further investigation. An attempt was made in some of the experiments, also, to determine the blood pressure in the hope of securing further light upon this change in pulse rate, but the results were not sufficiently satisfactory to permit positive deductions.

There are two methods by which the data may be studied to supply evidence regarding this change in pulse rate. One is to compare the records during the experiments with 20 per cent of oxygen with the records in the experiments with 90 per cent of oxygen. Due allowance should of course be made for the continual decrease in pulse rate which would naturally be expected with a subject lying upon a couch, but this may be done by comparing the days on which the experiments with 20 per cent of oxygen were made first with those on which those with 90 per cent of oxygen were made first. In making such a comparison we find that the records for the pulse rate when the subject was breathing atmospheres containing 90 per cent of oxygen were almost invariably lower than in experiments with an atmosphere containing 20 per cent of oxygen.

A considerable number of records of the pulse rate were made prior to and after experiments, while the subject was still quietly lying on the couch. An examination of these observations also supplies evidence regarding the change in the pulse rate occasioned by the change in the percentage of oxygen in the atmosphere breathed. These observations show that usually after the valve was thrown and the experiment proper began, the pulse rate noticeably decreased in the experiments with 90 per cent of oxygen, and returned to the normal rate when air with a content of 20 per cent of oxygen was again breathed at the close of the experiment. This may be seen in the records of a typical experiment given in Table VI, in which the figures in heavy type represent the records made during the experimental period. It was also noticeable that this decrease in pulse rate was very rapid, frequently being apparent in the first few minutes after the change to the oxygen-rich atmosphere was made. From all the records, therefore, which were usually made every three or five minutes, it is apparent that the inhalation of oxygen-rich gaseous mixtures, particularly those with a very high percentage of oxygen, results in a noticeable decrease in the pulse rate.

TABLE VI.

Pulse Rates of A. G. E. while breathing Gaseous Mixtures containing 20 per Cent or 90 per Cent of Oxygen, May 31, 1910.[1]

	Exp.									
90 per cent of oxygen	1	70	..	**69**	**62**	**64**	**62**	**61**	..	66
	2	66	73	**66**	**59**	**60**	**59**	**63**	**63**	..
	3	64	64	**61**	**62**	**63**	**59**	**61**	**60**	64
20 per cent of oxygen	4	68	66	**65**	**69**	**71**	**72**	**69**	**65**	..
	5	64	65	**70**	**70**	**69**	**71**	**71**	..	68

[1] The figures in heavy type represent the records taken during the experimental period, those in ordinary type being the records taken previous to and after the experiment.

Oxygen Therapy and Suggestions for Further Research.

While all of the experiments in this investigation were made with normal subjects, they throw certain definite light upon the rationale of using oxygen in therapy.[35] It would seem, *a priori*, to be impossible to increase the oxygen consumption of man by increasing the oxygen in the air inspired; but although hæmoglobin as an oxygen carrier is not noticeably affected by the inhalation of oxygen-rich mixtures, we must recognize the fact that the blood plasma contains a larger amount of oxygen which may be drawn upon, thus, at least temporarily, aiding slightly in making up any deficiency in the oxygenation by means of hæmoglobin. It is furthermore hardly probable that with bedside use of oxygen or in ordinary treatment we should have to deal with the oxygen pneumonia observed by J. Lorrain Smith[36] with small animals. The experiments made by this investigator lasted many hours, and we have no reason to believe that the use of oxygen for the short periods of the experiments here reported can in any wise induce disturbances of the respiratory tract. The noticeable influence on the pulse rate when inhaling oxygen-rich mixtures may also be of therapeutic importance.

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[35] Durig: Medizinische Klinik, 1905, Nos. 5 and 6, p. 1; Michaelis: Handbuch der Sauerstofftherapie, Berlin, 1906.

It is obvious, however, that many studies are needed in order to place oxygen therapy on a scientific basis. These investigations should include a study of the accurate determination of the oxygen consumption and carbon dioxide production when breathing oxygen-rich atmospheres in pathological cases, particularly pneumonia, cardiac disturbances, and tuberculosis. The influence of the inhalation of oxygen on the pulse rate, accompanied by a careful study of the blood pressure under different conditions, particularly in pathological cases, should also be carefully studied.

It is possible that in certain diseases in which the area for gaseous interchange in the lungs is markedly decreased as a result of consolidation or other causes, the oxygen-rich mixtures might prove of advantage from a mechanical standpoint, requiring less effort for respiratory ventilation on the part of the subject. The observations of Pembrey[37] seem to show conclusively that with the Cheyne-Stokes respiration the inhalation of oxygen-rich mixtures is advantageous, as the high percentage of oxygen raises the point at which carbon dioxide stimulates respiratory centres. We believe that the apparatus described here and the technique outlined may easily be adapted to most types of pathological experiments. The field for investigation is therefore very large and profitable results are almost assured.

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[36] J. Lorrain Smith: Journal of physiology, 1899, xxiv, p. 19.

[37] Pitt, Pembrey, and Allen: Medico-chirurgical transactions, London, 1907, xc, p. 79.

SUMMARY.

In experiments with normal individuals lying at complete muscular rest, twelve hours after the last meal, and breathing 40 per cent, 60 per cent, and 90 per cent oxygen mixtures, we find:

(*a*) that there is no apparent difference between the metabolism as indicated by the gaseous exchange (*i. e.*, the carbon dioxide output, oxygen consumption, and respiratory quotient) and the metabolism when breathing ordinary air;

(*b*) that there is no change in the respiration, either as to character, depth, or frequency, as compared with the same factors when breathing ordinary air;

(*c*) that the pulse rate is lower with oxygen-rich mixtures than when breathing ordinary air; furthermore, that the higher the percentage of oxygen breathed (up to 90 per cent), the lower the pulse.

A RESPIRATION APPARATUS FOR THE DETERMINATION OF THE CARBON DIOXIDE PRODUCED BY SMALL ANIMALS.

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THE early use of the determination of the carbon dioxide output as an index of total katabolism has led to the development of various types of apparatus, both for animals and man, for studying this factor of metabolism; in recent years such apparatus has been brought to a high degree of perfection.

The carbon dioxide production, however, does not always indicate the total katabolism with a satisfactory degree of accuracy, and in order to obtain the most accurate results, a determination of the oxygen consumption is frequently necessary. This is particularly the case when large variations in the proportions of carbohydrate enter into a diet, since we then have to deal with a fluctuating factor in the storage of body glycogen. Moreover, with men, consuming as they do a large variety of food materials, the use of the carbon dioxide production as an index of the total katabolism is especially unsatisfactory, as it is impracticable to feed a man on a carbohydrate-free, or, indeed, any constant diet for a considerable period of time. On the contrary, animals, particularly dogs, may be maintained on a constant diet, and under conditions of relatively constant muscular activity and temperature, for weeks, if not months. Under these circumstances, it is reasonable to assume that the variations in the store of body glycogen will not be appreciable; hence the carbon dioxide output of dogs or other animals, when obtained under proper conditions as to muscular activity and plane of nutrition, may logically be used, in lieu of a calorimetric estimation, as an index of the total katabolism.

Particularly is this the case if the factors to be studied are superimposed on a resting period and the amounts of carbohydrates ingested are never materially altered.[1]

After the animal has been maintained at a constant plane of nutrition for several days, if some factor affecting metabolism is brought into play, there will be an alteration in the regular metabolism which may be studied for as long a period as desired, the results being compared to the base line previously established.

It will thus be seen that much information can be secured and many problems settled with a reasonable degree of assurance by means of a careful, accurate measurement of the carbon dioxide production alone, thus doing away with time-consuming oxygen determinations and expensive calorimetric measurements. Such use of this factor is of especial value in making a preliminary survey of a field of research in metabolism. On the other hand, the fact should not be lost sight of that for a complete understanding of any particular factor influencing metabolism, oxygen determinations and, if possible, calorimetric measurements are highly desirable.

A contemplated study of the effect on dogs of the removal of the hypophysis, the injection of hypophyseal extracts, and the feeding of fresh or dried gland, led us to a careful inspection of the literature referring to the technique of making respiration experiments with these animals. This has brought us to the conclusion that sufficient attention has not heretofore been paid to maintaining the animal in comparable states of muscular activity at different periods of the experiment. While it is true that attempts have been made to secure for dogs temperatures high enough to prevent the muscular activity of shivering, yet, on the other hand, Lusk [2] has pointed out that, owing to the great variation in the metabolism of the dog at temperatures lower than 30° C., or thereabouts, a very large number of experiments reported as having been made at "room temperature" have, probably, no particular value.

Even with proper temperature conditions and the utmost care in training and preliminary experimenting, there is still evident in dogs an uncontrollable muscular activity. While it is true that Zuntz has been able to train dogs with a tracheal fistula to lie quiet for a considerable period of time, yet the great advantage of his method of studying the respiratory exchange, particularly with animals, depends upon the fact that such experiments can be made in short periods, during which the extraneous muscular activity is eliminated. Although it is perfectly practical to prepare tracheal fistulas in dogs, and indeed in large animals, as Zuntz has done, this technique is very often difficult to carry out when other experiments on the same animal are contemplated; hence observers have, as a rule, been content to utilize either the Voit respiration chamber or a modification of the closed chamber in which the animal remains quiet and the carbon dioxide accumulates.

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[1] The errors incidental to ingesting carbohydrates when the body has been for some time upon a carbohydrate-free diet or after inanition have been clearly pointed out recently by ZUNTZ: Naturwissenschaftliche Rundschau, xxi, No. 38, p. 2.

[2] LUSK: Science of nutrition, Philadelphia and London, 1909, p. 153.

By far the largest number of experiments on dogs have been made by the investigators of the Munich school, notably Rubner, in which the Voit respiration chamber has been used. In this apparatus a current of air is passed through a chamber, measured by means of a metre, and samples taken through a smaller metre. By passing the sample of air through a barium hydroxide solution, the carbon dioxide is absorbed, and the amount determined by titration. The ventilation of the chamber can thus be adjusted so as to keep the carbon dioxide content down to a moderate percentage.

Frequent use has also been made of a closed chamber [3] in which the carbon dioxide has been allowed to accumulate.[4] The increment in carbon dioxide, determined by the analysis of air samples, thus furnishes the measure of the total carbon dioxide produced. This method involves a careful measurement of both the volume of the chamber and the temperature of the air inside the chamber, together with records of the barometric pressure and moisture content. The most important feature of the method, however, is the analysis of the gas samples. For this, Chauveau and Tissot have employed an exceedingly delicate and fragile gas analysis apparatus devised chiefly for blood gas analyses, but this requires very great skill for its successful use.[5]

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[3] KAUFMANN: Archives de physiologie, 1896, (5), viii, p. 329; LAULANIE: Archives de physiologie, 1894, (5), vi, p. 845.

[4] LAULANIE (*Loc. cit.*) has shown that disturbances in metabolism are not noted in small animals until the carbon dioxide has increased to 6 per cent or the oxygen decreased to about 11 per cent.

BENEDICT and MILNER (United States Department of Agriculture, Bulletin 175, 1907, p. 256), working with man, found the same carbon dioxide production, oxygen consumption, and heat elimination when the man was breathing an atmosphere containing 2 per cent of carbon dioxide as when breathing normal air.

In connection with our respiration experiments on dogs, we have adopted a modified form of respiration apparatus which has proved so satisfactory and has brought out so many interesting points that it has seemed of value to give a description of it here. The apparatus in question is a closed chamber in which the carbon dioxide content of the air is allowed gradually to rise, the air in the chamber being thoroughly mixed by an electric fan. At the end of each experimental period, which may be thirty, forty-five, or sixty minutes in length, gas samples are taken and subsequently analyzed.

THE RESPIRATION CHAMBER.

We first constructed a chamber with a capacity of approximately 1000 litres. For the purpose of controlling the temperature within reasonable limits, the chamber was provided with a coil of brass pipe through which cold water could be passed to bring away the heat produced by the animal as fast as it was formed. Subsequent experience has shown that this rise in temperature can readily be controlled by cooling the laboratory room, particularly during the winter. This first apparatus was of a preliminary nature, and it was soon seen that for the size of the dog used (5 kgm.) the chamber was too large. Hence a much smaller apparatus was subsequently constructed, in which a number of important changes were made as a result of our experience with the first chamber.

The respiration chamber finally employed has a dimension of approximately 60 cm. on each side. It is constructed of galvanized sheet iron, all of the seams being well reinforced and soldered so as to be air-tight. The general structure of the apparatus and accessories is shown in Fig. 1. The top, which is removable, is provided with a flange which can be lowered into a water seal. This water seal is provided by a small trough, *H*, *H*, soldered firmly to the upper part of the sides of the chamber. When the cover is in place, if sufficient water is poured into this trough, a perfect closure is secured. While oil is theoretically better than water for such a seal, it has the disadvantage of being very dirty and liable to become distributed all over the apparatus. No valid objection can be raised to the use of water as a seal in this apparatus, since the determination of the water vaporized by the dog was not attempted; furthermore, the possibility of the absorption of carbon dioxide by the small surface of water exposed is so slight that it cannot seriously affect the results. A glass window, *W*, 16 cm. square, is inserted tightly in the centre of the top,[6] thus giving an opportunity for the direct observation of the dog below.

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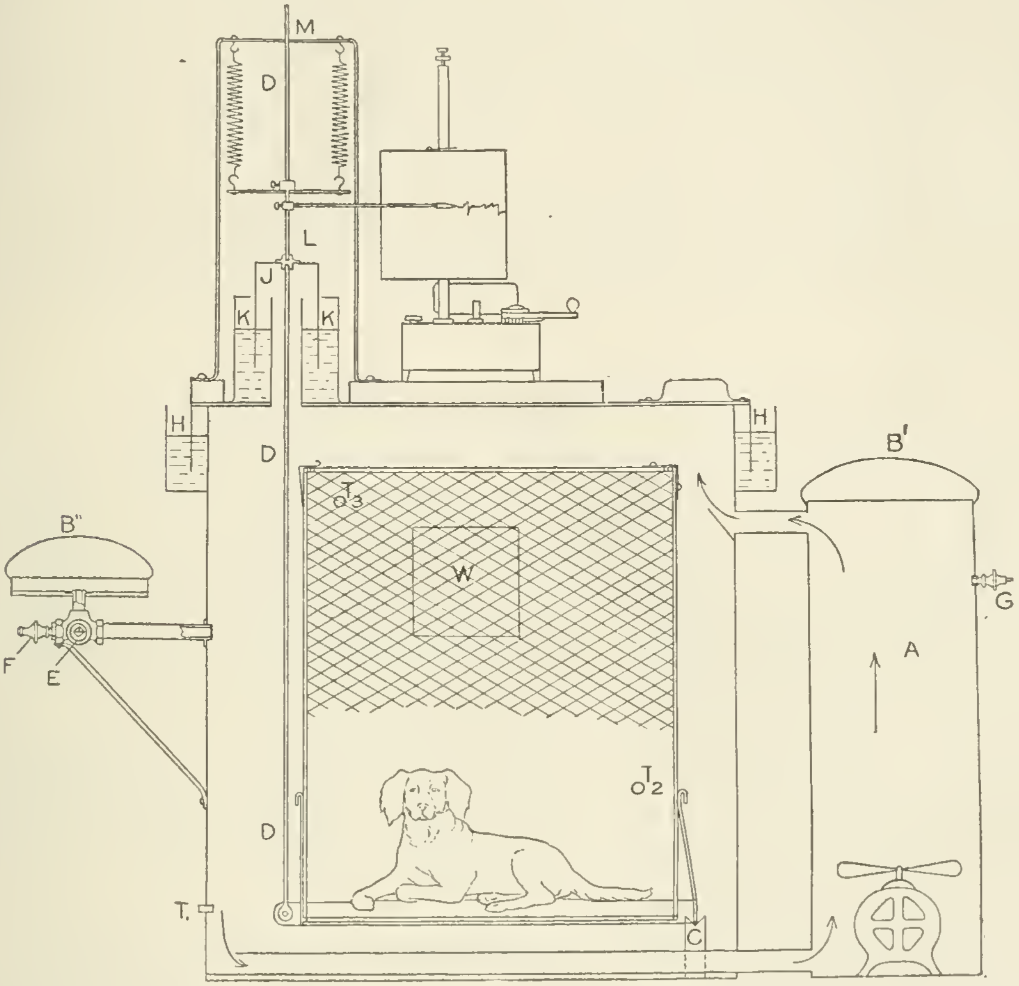
[5] LAULANIE (*Loc. cit.*) reports a brilliant series of analyses carried out by means of a eudiometer, described by him in Archives de physiologie, 1894, (5), vi, p. 739.



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[6] In the figure this is shown for convenience on the side of the chamber rather than on the top.

On one side of the apparatus is attached a vertical sheet iron pipe, *A*, 22 cm. in diameter, in which is placed an electric fan. This pipe, which is connected with the chamber at the bottom, is carried across the floor so that the opening is at the farther side. A complete circulation of air is thus provided by the current which is drawn in at the bottom and blown into the top of the chamber through a second opening. In order to make the fan accessible for repairs or for alteration, the outside opening in the top of the cylindrical pipe is closed by a rubber bathing cap, B'; this allows considerable expansion of the air inside the chamber, and facilitates the measurement of the volume as well as the regulation of temperature control. Air samples for analysis are withdrawn at the petcock, *G*, the point of most complete mixing of the air.

On the opposite side of the chamber is attached a brass pipe supporting a shallow tin pan covered by a rubber bathing cap, B'', which is connected with the chamber by the 3-way petcock, *E*. This diaphragm allows for the expansion and contraction of the air inside of the chamber. The petcock *F* may be used to introduce oxygen if metabolism in oxygen-rich atmospheres is to be studied. At different points about the sides of the chamber are attached tubes, T_1, T_2, and T_3, through which thermometers can be inserted. By using the electric fan to mix the air in the chamber, not only the composition but also the temperature of the air is made relatively uniform, so that temperature observations in different parts of the chamber usually show no material differences.

The total volume of the chamber is approximately 280 litres, and each bathing cap can be filled by distention so as to include 2.5 or 3 litres more of air. Inasmuch as it is possible to judge the height of the bathing caps very closely by the eye, it has been deemed inadvisable to attempt to calibrate the height of the diaphragms and obtain the exact volumes therein, as with an error of even 2 litres the error in measurement of the volume of the chamber would be inside of 1 per cent. Marked changes in temperature should, however, be taken into consideration, as well as any considerable fluctuations of the barometer.

In our experiments with dogs, it was soon found that excessive moisture conditions did not prevail. The dog did no muscular work in any of the experiments, and at no time was there a condensation of moisture observed on the glass window. While theoretically, for the most exact work, calculations should be made of the total volume of nitrogen plus oxygen plus carbon dioxide, *i. e.*, the volume of dry air in the chamber, this is not necessary in the majority of instances. Probably the best procedure is that of Kaufmann,[7] who insured saturation of the air by suspending wet cloths inside the chamber.

The temperature control of the apparatus is usually readily made by cooling the laboratory room, usually by opening the window. Indeed, this temperature control is so easily made that in constructing the smaller apparatus the coil of cooling pipe was omitted, as its use would have diminished the space inside the chamber.

CONTROL TESTS OF THE RESPIRATION CHAMBER.

Method of testing the chamber for tightness. — By a simple method this chamber is very readily tested for tightness. Sufficient pressure to inflate the bathing caps is first introduced into the chamber by means of a bicycle pump or the service of compressed air. A small weight is then put upon the bathing caps and the height noted. A leak is very quickly indicated by the sinking of the rubber bathing caps, as the loss of 100 c.c. in a half hour can be readily observed, due allowance being made for fluctuations in temperature and in the barometer. As under experimental conditions, there is no measurable pressure inside the chamber, any leak involving a change in volume of not more than 100 or 200 c.c. in half an hour can be neglected.

Alcohol check tests. — With a large respiration calorimeter it is relatively easy to make alcohol check tests, but with this apparatus it has been found more difficult. Naturally the best tests are those in which the conditions approximate as closely as possible the conditions of the experiments. The ideal method would be, therefore, to develop carbon dioxide inside the chamber at the rate commonly shown by the dog. For such check tests a form of alcohol lamp elsewhere described[8] has been used. When, however, the carbon dioxide is allowed to accumulate by this method, a difficulty arises in that a point is soon reached when the flame is affected, and only a slight increase in the percentage of carbon dioxide in the air is sufficient to extinguish it. Preliminary tests showed that this effect upon the flame was due as much to the decrease in the oxygen percentage as to the increase in that of carbon dioxide. Accordingly an attempt was made to overcome this difficulty by filling the apparatus with an oxygen-rich atmosphere before beginning the tests. This was done by first placing the cover on the chamber, and then introducing oxygen from a cylinder through the petcock, *F*, until the desired oxygen percentage was obtained. With this oxygen-rich atmosphere it was found that the alcohol burned freely, so that a test covering several hours could easily be carried out. At the end of the experiment samples were taken and analyses made as usual.

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[7] KAUFMANN: *Loc. cit.*

[8] BENEDICT, RICHE, and EMMES: This journal, 1910, xxvi, p. 7.

Alcohol check tests with this apparatus have shown that the results are slightly affected by the temperature and by changes in barometric pressure; humidity also unquestionably plays a rôle. While we have tentatively estimated the error in the determination of carbon dioxide to be 3 per cent, with refinement of temperature control, exact care in analysis, and in the barometric and hygrometric observations, this error should be somewhat, if not considerably, decreased.

It should be stated here that, although it has not been attempted as yet in this laboratory, there appears to be no reason why the apparatus cannot be used for measuring the oxygen consumption of an animal as well as the carbon dioxide production. Laulanie's [9] experience with this type of apparatus apparently leaves little to be desired, but instead of the cumbersome eudiometer employed by Laulanie, a Haldane gas analysis apparatus can be used, as with it very accurate determinations of oxygen can be made. As the increment in the carbon dioxide percentage and the decrease in the oxygen percentage can be measured with approximately equal accuracy, there should be no great difficulty in determining the respiratory exchange. In that case the most exact measurements of the temperature and of the barometer should be made, and probably more attention would have to be paid to the moisture content of the air inside the chamber.

ANALYSIS OF AIR SAMPLES.

For the analysis of the air samples we have employed the Haldane gas analysis apparatus.[10] Although Haldane makes his analyses over mercury, we have found it advantageous to take samples of the air over water, and, indeed, in the tourniquet apparatus so frequently used by Zuntz. This apparatus consists of six long, narrow glass tubes with a capacity of 340 c.c. each, these tubes being so arranged that they are supplied through their lower ends with water from a common reservoir, the water being acidulated with hydrochloric acid, as recommended by Zuntz. Clamps are placed upon the rubber tubing at the upper and lower ends, so that by raising or lowering the water reservoir (a light glass bulb) each tube can be separately filled with air and emptied.

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[9] LAULANIE: *Loc. cit.*, p. 845.

[10] HALDANE: Journal of physiology, 1898, xxii, p. 465.

The Haldane apparatus is very easy to use, and extremely accurate results can be rapidly obtained. The one objectionable feature is the calibration of the pipettes. At present, as no manufacturer furnishes these pipettes with suitable accuracy, it is necessary to make one's own calibrations. The calibrations are somewhat difficult to make, since in drawing off the mercury it is necessary to attach a jet or glass stopcock to the lower end. The tall column of mercury, nearly 85 centimetres in height, produces a great pressure on the rubber connection below, distending it somewhat; as the level of the mercury falls, the rubber contracts, thus producing errors in the calibration. Haldane has suggested that this difficulty may be overcome by inverting the burettes and drawing the mercury off by the glass stopcock at the top; in that case an additional correction is necessary to allow for the meniscus of the mercury. Haldane has frequently had a special glass stopcock temporarily fused to the lower end of the burette until this calibration has been made.

APPARATUS FOR INDICATING THE MUSCULAR ACTIVITY OF THE ANIMAL.

Inasmuch as it is usually impossible to obtain with the subject of the experiment a condition of absolute muscular rest, a record of the dog's muscular activity is very important. Even with the glass placed in the cover of the box, it is impracticable at every moment to watch the animal closely and to record any extraneous movement. Furthermore, it is almost impossible to give a mathematical expression to the intensity of these movements. Even with an animal well trained to remain very quiet, there are almost invariably slight muscular movements, which, though apparent to the eye, do not appear to be of very great moment. Nevertheless, when these movements are graphically recorded and the metabolism in the periods in which such movements occurred is compared with that in the periods in which such movements did not occur, the great desirability becomes apparent of knowing accurately the intensity of this activity. The importance of these movements in contributing to the metabolism in any given periods is therefore obvious.

A graphic record of the activity of the animal experimented upon has been obtained with this apparatus in the following manner: The animal is confined in a cage which is placed upon a hanging shelf or rack, one end of which rests on a support, *C*, attached to the floor of the calorimeter chamber. A knife-edge bearing minimizes friction. The other end of the shelf swings free, being suspended by a rod, *D*, *D*, *D*, and spiral springs from a support, *M*, above the chamber. At the upper end of the rod is attached a pointer which records on the smoked paper of a kymograph any movements of the rod. As this rod passes through the cover of the apparatus, an air-tight closure is necessary, and for this we have used a cylinder, *J*, open at the lower end and screwed to the rod at *L*. This cylinder dips into water in an annular space, *K*, *K*, between two cylinders soldered to the top of the cover of the chamber. This provides an air-tight closure. As the rod moves, the cylinder plays up and down in the water with a minimum amount of friction.

Obviously, with the cage suspended in this manner, any change in the position of the centre of gravity of the dog will disturb the equilibrium between the end of the rack fastened to the support on the floor of the chamber and the end suspended by the spring, and thus greater or less tension will be thrown upon the spring, causing the rod to move up and down. This movement of the rod is recorded on the smoked paper, and it is possible in this way to secure very satisfactory tracings of the muscular movements of the dog. All muscular movements that are noticeable to the eye are instantly recorded, and even lesser activity, such as slight shivering, can be detected. As the tension can be arbitrarily adjusted so as to permit of larger or smaller fluctuations in the movements of the spring, any desired magnification of the record can be obtained by adjusting the sensitiveness of the spring. If the dog for any reason makes a major motion and shifts the position in which he lies, and then subsequently lies quiet, the pointer simply adjusts itself to another level on the drum of the kymograph, and the line will thereafter remain straight as long as the dog is quiet. Naturally the best results are obtained by having the weight of the cage and support as small as possible, and this has been taken into consideration in the construction of the apparatus. Sample records of the muscular activity of a dog obtained by this method are reproduced in curve 1.

Description of Kymograph Curve showing Muscular Activity.

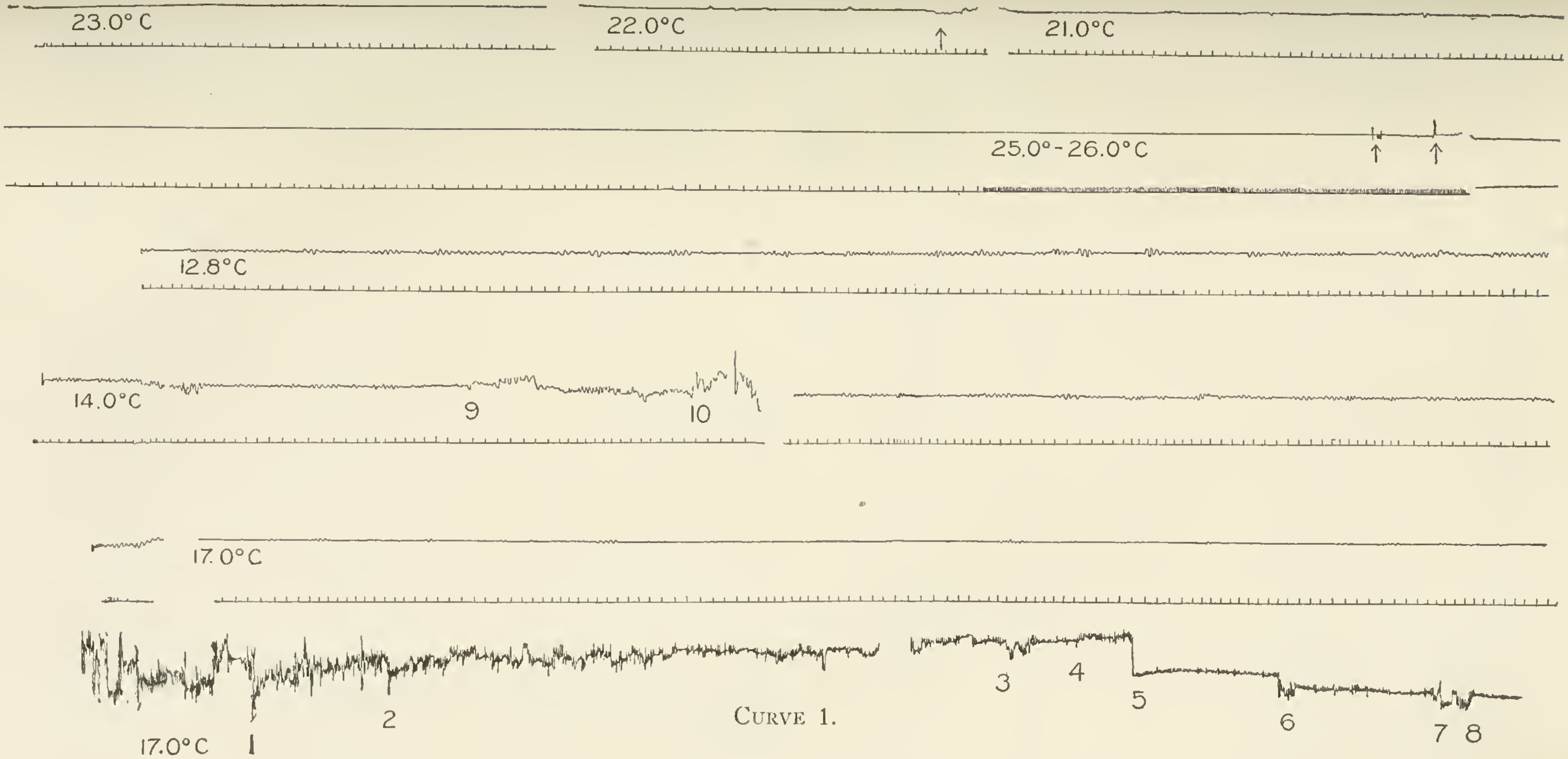
The curve should be read from the bottom to the top. For obtaining these records, the cage was suspended outside of the regular chamber so that observations of the dog could be made which could be compared later with the record of the fluctuations of the pointer. Exactly the same knife edges and bearings were used and the exact relation of all parts was retained, the only difference between this test and a regular experiment being that the enclosing wall of galvanized iron was not employed. Inasmuch as this was an abnormal situation for the dog, he did not settle down for some time after he was placed in the cage. This period of settling down is very well shown in the lowest record. It should be borne in mind that each of these movements represents a vertical displacement of the cage, and the amplitude of vibration is exactly twice the vertical displacement of the centre of the cage. As the kymograph drum rotated, notes were made at different points of observations regarding the position of the dog. These follow:

1. Dog sat up on haunches.
2. Partly stood up for a moment and then sat down instantly.
3. Sitting with left fore-paw bent and the head over the back.
4. The head resting on the back.
5. Lay down with the head over the back, well toward the tail.
6. Head withdrawn, and nose placed under hind leg.
7. Head up, looking about.
8. Head under leg, in perfect repose.

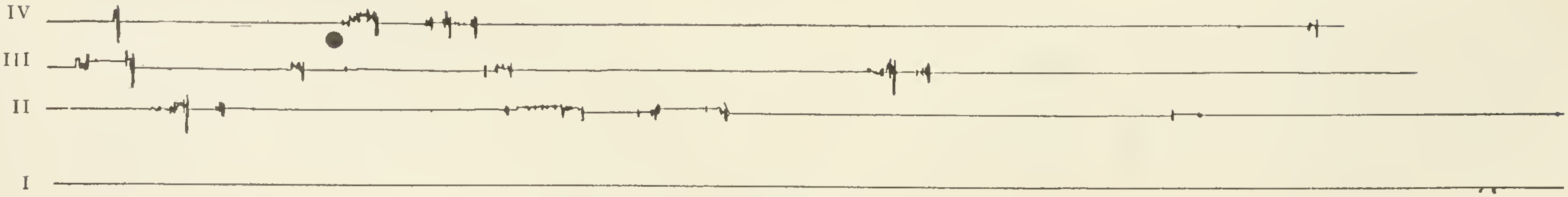
The temperature at this time was 17° C., and in order to make a more careful analysis of the minor fluctuations at this temperature the speed of the kymograph was greatly accelerated and a Jaquet clock for marking seconds was used to take the subsequent record. The second record (the first one with time markings below it) indicates that the dog remained perfectly quiet except for shivering, which would be noticeable at a temperature of 17° C. The window was then opened, and the room allowed to cool. When the temperature had reached 14° C., a record with a rapidly moving drum, accompanied by a time record in seconds, was likewise taken. According to this record, the dog was somewhat restless, as, beginning at the point marked 9 on the 14° C. curve, he moved his head, then sat partly up, and lay down again immediately, finally at 10 assuming a position of rest, aside from shivering. The marked accentuations of the shivering can be seen as well as the rhythmical movement of the line. This rhythm is due to the respiration, which movement is superimposed upon the regular shivering. When the temperature of the room had reached 12.8° C., another record was taken; this shows even more clearly the marked effects of shivering, with the superimposed respiration movement. The room was then rapidly heated to 25° C. and with a rapidly moving drum a record was taken for several minutes, resulting in an absolutely straight line. After two or three minutes the drum was slowed down and a further record taken for ten minutes. At two points indicated at the latter end of this record, the dog moved his head twice. The temperature was then gradually lowered and short records taken at 23°, 22°, and 21°. At 22° C. we begin to find slight movements of the dog, although these slight shivering movements are obscured at the end of the record by a movement of the head. At 21° the slight movement is still more noticeable than at 22°.

One important deduction which may be drawn from this kymograph curve is that the records prove true what has been found by many experimenters from practical experience, *i. e.*, that a temperature of 25° C. or above is necessary for absolute quiet on the part of the dog. This dog was short-haired, and a different set of records might have been obtained with a long-haired dog. It is very easy, however, with records of this kind to find what temperature is best suited for absolute quietness on the part of the animal experimented with.

It should be stated that this method can also be used to determine the muscular activity of other animals, such as rats, mice, or rabbits,



Curve 1.



Curve 2.

by placing them in small metabolism cages either inside or outside of a respiration chamber, and attaching the cages to levers as in the apparatus here described.[11]

As an index of the extreme closeness of the relationship between the muscular activity and the carbon dioxide production, we show in the table the carbon dioxide production per forty-five minutes in an experiment with a dog, and also in curve 2, the kymograph records obtained in the same experiment.

KYMOGRAPH RECORD OBTAINED IN A METABOLISM EXPERIMENT ON A DOG.

The record [12] shown in curve 2 was obtained in a regular metabolism experiment made with a dog on May 11, 1910. It should be stated that the apparatus was at that time in the earlier stages of development, and the cage used was very much heavier than that employed when the records in curve 1 were made. As a matter of fact the cage used on May 11, 1910, weighed 11.6 kgm.; the one used for the records in curve 1 weighed but 3.4 kgm.; the weight of the

CARBON DIOXIDE PRODUCTION IN METABOLISM EXPERIMENT WITH DOG (MAY 11, 1910).

Period.	Carbon Dioxide Residual.				Carbon dioxide produced.
	Percentage.		Amount.		
	At beginning.	At end.	At beginning.	At end.	
	per cent	per cent	gm.	gm.	gm.
I	0.262	0.791	1.283	3.872	2.59
II	0.791	1.404	3.872	6.873	3.00
III	1.404	2.028	6.873	9.928	3.06
IV	2.028	2.592	9.928	12.689	2.76
Total					11.41

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[11] At the moment of writing, this principle is being applied to the larger calorimeters for man installed in the Nutrition Laboratory.

[12] At the time this record was taken the temperature of the apparatus rose slowly from 21° at the beginning to 23° at the end.

dog was 5 kgm. Consequently the vibrations are much diminished and the sensitiveness of the apparatus much reduced.

These records are again to be read from the bottom up. It can be seen that during the first period the dog was apparently absolutely quiet. In the other periods there were motions which in all probability were due to the lifting up of the head and keeping it raised for several moments. When the dog is lying curled up, as is usually the case during an experiment, the effect upon the metabolism of raising the head may be somewhat more than that of the simple muscular activity. There is a certain part of the skin area, *i. e.*, the lower part of the neck and part of the thigh and body, which is warmed when the head is placed closely against it. When the head is raised, not only is this muscular activity involved, but there is likewise a cooling off of the skin which must subsequently be warmed. It may readily happen, therefore, that in a period where several head movements follow each other rapidly, the metabolism may be less than in a period where the head movements were quite separated one from the other. It is on this basis that we explain the rise in carbon dioxide found in period II,[13] as shown in the table.

For example, an examination of the table would indicate that the muscular activity was apparently no greater in period II than in period IV, and yet it can be seen upon a close examination that the restlessness continued throughout the whole of period II to a greater or less extent, while in period IV the restlessness was chiefly in the first part of the period, with a slight restlessness just at the end, probably too near the end to be measured with the sample of that period.[14] The value of the records, however, for interpreting the variations in metabolism is self-evident.

In studying any particular factor in metabolism, the assumption that an increase in the carbon dioxide production is due to the superimposed conditions is manifestly erroneous unless the extraneous muscular activity is taken into consideration. With a series of graphic records of the muscular activity of the subject, it is possible to select periods in the experiment in which the muscular activity was at a minimum, and no major muscular movements occurred. If the metabolism for these periods is compared with the base line, we have a much more scientifically sound basis for comparison than can be obtained in any other way. It is believed that the principles and the method here outlined are capable of very great extension and should be of great service to physiologists in experiments with animals.[15]

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[13] An examination of the curve shows that the centre of gravity of the body of the dog was frequently changed sufficiently to indicate a noticeable difference in the levels of the record on the smoked paper.

[14] The taking of the sample at the end of each period was coincident with the end of the line on the record.

ROUTINE OF EXPERIMENTS.

For the purpose of collecting the urine and feces, the animal is kept in a metabolism cage of any ordinary type, which is placed in a room with good light and with as uniform a temperature as possible. The animal is fed once a day, always in the afternoon, the amount of food being weighed, and the amount of water measured. In order to control the accuracy of this proceeding, a sample of food, similar to that given to the dog, is analyzed once a week. A given amount of water is mixed daily with the food, and in addition a known quantity is given to the animal in a dish; the amount the dog drinks is thus easily estimated. If any of the food given to the dog is left uneaten, it is removed, but this is and should be of rare occurrence, for the amount of uneaten food can be only roughly estimated. The experiments are always made in the morning, approximately sixteen hours after feeding.

Daily observations of the animal. — On being taken from the cage, the dog is first weighed, then allowed to lie still in the observer's lap, while records of the pulse, respiration, and temperature are being taken. For accurate observations of these factors, the animal should be completely relaxed and quiet, and this state may not be reached for fifteen to thirty minutes; even after the animal has become quiet, the pulse rate may continue to fall for five or ten minutes.. The slightest shivering or spasmodic respiration increases the pulse and respiration rates materially. This shivering is apparently due to cold, and though different dogs vary, and even the same dog may react differently from time to time to low temperatures, it is almost always necessary with short-haired dogs to secure a room temperature of at least 24° C. in order that the animal may be thoroughly relaxed. The mimimum constant pulse and respiration rates are of course taken.

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[15] A modification of the details of this apparatus has recently been made by us in which the movements of the animal cage are transmitted by means of a Fitz tube pneumograph to a Marey tambour outside of the respiration chamber. Preliminary tests with this modification lead us to believe that much may reasonably be expected of it. The apparatus described in this paper has, however, been used for an extensive series of experiments, the results of which are now being prepared for publication.

THE RESPIRATION CHAMBER PERIOD.

After the records of the pulse, respiration, and temperature have been obtained, the animal is at once placed in the chamber, the fan is started, and the cover closed down. If the temperature is not at least 24° C. at the beginning of the experiment, it is raised by any convenient means until this point is reached.[16] Until the dog lies quietly and breathes without muscular twitchings, no sample is taken. During this preliminary period, which may often require from half an hour to an hour, the movements of the animal are recorded on the drum. When the record shows the dog to be quiet, and the temperature has been satisfactorily adjusted, the first sample is taken.

Several minutes before the time for taking the first sample, the petcock, *G*, is connected by a short capillary glass tube with one of the sample tubes, which has previously been filled with room air. As the withdrawal of the volume of air required for a sample would reduce the volume of air in the system, this is compensated for by introducing into the chamber the entire air content of the sample tube by lifting the reservoir, and then in the same way alternately filling and emptying the sample tube with air from the chamber. For the next few minutes, or until the exact moment of taking the sample, the tube is several times filled and emptied of air from the chamber. Accordingly, when the sample is finally withdrawn, it represents the most complete mixture obtainable. As the actual filling of the tube occupies only fifteen to twenty seconds, the timing of the operation can be made sufficiently accurate.

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[16] The methods generally used have been to raise the temperature by letting the sunshine fall upon the chamber, or by placing a gas stove near it; and to lower the temperature by opening the windows, creating a draught, etc. With but rare exceptions, the temperature is kept between 24° and 25° C.

The sample is now taken to the Haldane apparatus and immediately analyzed. Test experiments have shown that if the tube is completely filled with air, so that only a little water is left adhering to its sides, the sample may be allowed to stand for several hours before being analyzed. On the other hand, if one analysis is made, in which the water is allowed to rise in the sample tube, succeeding check analyses must be made at once, or the water will dissolve an appreciable amount of carbon dioxide. The practice has therefore been followed of making a duplicate analysis of each sample before the next is obtained.

During the experiment all noise, except that necessarily made by the observer, is, so far as possible, excluded. Samples of air are usually taken every thirty to forty-five minutes. If at the end of two periods the drum record shows that the animal has been quiet, he is often removed and the experiment concluded.[17]

If during one or more periods the dog has moved considerably or if for any reason there is doubt about the accuracy of the sampling, the animal is kept in the chamber until a satisfactory series of periods is obtained, or until it is considered desirable to abandon the experiment. During this time the temperature inside the box is constantly recorded and if necessary regulated. The dog is then removed and, as a control, records of the pulse, respiration, and rectal temperature are again taken.

THE USE OF KYMOGRAPH RECORDS.

Whenever the dog is at all restless in the chamber, the kymograph record gives an accurate account of the extent of his movements. By comparing the rate of increase in carbon dioxide during the successive periods of any one experiment with the writings on the drum, the effect of the exercise upon the gaseous metabolism can readily be seen. In order to compare the carbon dioxide production in a series of experiments, it is absolutely necessary to select for comparison periods in which the record shows the activities of the animal to have been practically identical.

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[17] The rate of carbon dioxide production is not affected by its accumulation in the chamber in quantities under 6 per cent. LAULANIE: *Loc. cit.* In this series of experiments it has not been carried beyond 3.5 per cent.

RELATION OF PULSE RATE TO CARBON DIOXIDE PRODUCTION.

The experience on men in the Nutrition Laboratory[18] indicated the importance of studying the relation between pulse rate and metabolism on animals.

For the purpose of checking the experiments on carbon dioxide production, accurate daily observations of the pulse and respiration rates have been made. It is plain that unless these observations are made daily and under identical circumstances, they will be of little value. During several months' work the possibilities of inaccuracy in this direction were developed, especially such inaccuracies as might result from the chance excitation of the animal, improper temperature, accidental feeding, etc. In connection with this work an attempt was made to take a daily blood pressure observation, by means of a Leonard Hill sphygmometer. This accentuated the well-known fact that no satisfactory method has as yet been devised for taking the blood pressure of a small animal without the direct use of an artery. It was found that upon such a small animal our observations were necessarily inaccurate, and the attempt was accordingly abandoned. The pulse rate alone, however, has proved a fairly accurate index of the carbon dioxide production, — always providing that the conditions of muscular activity under which the two records are made are identical.

SUMMARY.

1. The closed chamber method for determining carbon dioxide in short experiments with small animals has been resuscitated. It is strongly to be recommended for a preliminary survey of many research problems in animal metabolism, but is not offered as a substitute for direct determinations of oxygen and heat.

2. Samples of the confined air in the chamber are analyzed by the exceedingly exact and relatively simple apparatus and method of Haldane.

3. Graphic records expressing with approximate mathematical accuracy the variations in the muscular activity of the animal are

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[18] BENEDICT and CARPENTER: Carnegie Institution of Washington Publication No. 126, p. 248.

obtained by suspending the animal cage on a movable rack connected with supporting springs on the top of the chamber. A pointer records on a smoked paper kymograph the vertical displacement of this cage due to alteration in the position of the centre of gravity of the animal.

4. A description is given of the routine found best adapted for metabolism experiments with dogs with this apparatus.

A QUANTITATIVE STUDY OF FARADIC STIMULATION. — VI. THE COMPARISON OF ONE INDUCTORIUM WITH ANOTHER.

By E. G. MARTIN.

[*From the Laboratory of Physiology in the Harvard Medical School.*]

IN developing a system for expressing the strengths of induction shocks in terms of "stimulation units" it is of course essential that the method of obtaining these units be applicable to various inductoria, so that a given strength of stimulus shall be expressed by the same figures no matter by what inductorium the stimulus is generated.

The method employed in this work for making different inductoria comparable consists in introducing into the expression for the strength of stimulus a factor L which depends upon the construction of the secondary coil,[1] it having shown experimentally that by the introduction of this factor in the proper way corresponding stimuli give corresponding values in terms of stimulation units when generated by different inductoria.[2] Recently, however, Gildemeister,[3] working with inductoria made purposely to differ widely from each other in construction, has pointed out that in such inductoria the comparison of one with another depends in part upon the resistance in the secondary circuit, so that the simple introduction of the factor L into the expression for stimulation strength is not sufficient to make all stimuli directly comparable. This observation of Gildemeister makes necessary a re-examination of the method of comparing inductoria quantitatively in order that proper account may be taken of the influence of secondary resistance on the comparison.

Gildemeister's work shows that if two dissimilar inductoria are compared quantitatively, by the method outlined in this series of papers, in which the expression for the value of a stimulus is $Z = \frac{M}{L} \times I$,[4] it will be found that although equal stimuli may yield corresponding values of Z from the two inductoria with certain secondary resistances, when other secondary resistances are used equal stimuli will not give corresponding values of Z.

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[1] MARTIN: This journal, 1908, xxii, p. 120.

[2] MARTIN: *Loc. cit.*, p. 127.

[3] GILDEMEISTER: Archiv für die gesammte Physiologie, 1910, cxxxi, p. 604.

In a recent paper of this series,[5] the influence of secondary resistance on stimulating effectiveness was studied with reference to a single inductorium. In that paper it was shown that the actual or specific stimulus may be computed from the observed stimulus by the equation $\beta = \frac{Z\,A}{R + A}$, β being the specific value of the stimulus, Z its observed value, R the secondary resistance, and A a constant depending on the kathode surface.

In the same paper (p. 231) a method of determining the value of A experimentally was described, the expression for A being

$$A = \frac{Z_R R' - Z_{R'} R}{Z_{R'} - Z_R},$$

in which Z_R and $Z_{R'}$ are stimuli which with resistances R and R' respectively have equal physiological effect. Since, as stated above, dissimilar inductoria fail to give corresponding values of Z at all secondary resistances, it is clear that the value of A determined by this formula from one inductorium will not necessarily agree with its value as obtained from another. The value of A, therefore, does not depend solely upon the surface of the physiological kathodes as assumed previously,[6] but in part also upon the construction of the inductorium.

This variation in the values of A determined from dissimilar inductoria, which might lead one to question the validity of the equation in which A is employed, *i. e.*, $\beta = \frac{Z\,A}{R + A}$, serves in fact to confirm strongly the validity of that equation and the use of the expression

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[4] For the significance of the factors making up this expression, see This journal, 1908, xxii, p. 119.

[5] Martin: This journal, 1910, xxvii, p. 226.

[6] Martin: *Loc. cit.*, p. 230.

β to signify the specific value of the stimulus. This confirmation rests upon the repeated observation that when equal stimuli are generated by dissimilar inductoria the *values of β are equal*, even though the observed values of Z and the computed values of A may be quite divergent. An experiment illustrating this point is summarized in Table I. Details of the construction of the inductoria used are given in Table II.

TABLE I.

DEMONSTRATING THAT EQUAL STIMULI GIVE EQUAL VALUES OF β IN THE EQUATION $\beta = \frac{ZA}{R + A}$, WHEN THE STIMULI ARE GENERATED BY DISSIMILAR INDUCTORIA.

Inductorium.	H.	N.	B.
First sec. resistance .	8850 ohms	9000 ohms	9800 ohms
First Z	0.76	0.604	0.588
Second sec. resistance	25,500 ohms	25,600 ohms	26,400 ohms
Second Z	1.60	1.18	1.06
Calculated A . . .	6200	8700	10,400
Calculated β . . .	0.31	0.30	0.30

The differences in secondary resistance in corresponding columns are due to the different resistances of the secondary coils, it being necessary to include these resistances as part of the secondary circuit.

The observation just cited shows that even dissimilar inductoria give for equal stimuli perfectly concordant values if all the factors which make up the final expression for stimulation strength are taken into account. In a preceding paper,[7] however, I have acknowledged the great technical difficulties involved in including all the factors, and have shown that in many sorts of experimental work it is by no means necessary to take into account the factors of secondary resistance and kathode surface. Since it has been shown in this paper that the structure of the inductorium is interwoven with these factors, it becomes necessary either to admit that they must always be taken into account or to work out a method whereby it shall be unnecessary to take account of the influence of inductorium construction upon them. Fortunately a simple means exists for accomplishing this latter result.

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[7] MARTIN: *Loc. cit.*, p. 232.

It will be remembered that it is by the use of *dissimilar* inductoria that the interrelation between kathode surface and inductorium construction as factors modifying stimulation strength has been shown. By adopting a standard of inductorium construction and using for quantitative purposes only instruments conforming reasonably to the standard we become at once independent of inductorium structure as a complicating factor, and are free to measure stimuli in the simpler manner previously urged.

The desirability of having a standard of inductorium construction for physiological and clinical use was recognized fully thirty years ago. In an attempt to establish one the Paris Electrical Congress of 1881 resolved at its session of September 28 that the form of inductorium at that time in use in the University of Berlin should be adopted as the standard.[8]

The dimensions of that inductorium are as follows:

	Primary.	Secondary.
Length of coil	88 mm.	65 mm.
Diameter of coil	36 mm.	68 mm.
Diameter of wire	1 mm.	0.25 mm.
Number turns of wire	300	5000
Number layers of wire	4	28
Resistance	1.5 ohm.	300 ohms.

Unfortunately for the general acceptance of these dimensions as standard, Kronecker [9] had, ten years earlier, proposed his well-known system of units, based on determinations made with inductoria having coils twice as long as the Berlin coil and each with twice as many turns of wire. With the adoption of his graduation the large coils came into common use. By general consent among physiologists, therefore, rather than by any official action, inductoria having coils about 13 cm. long and having about 10,000 turns in the secondary are recognized as suitable for the uses of the investigator. In most well-equipped laboratories such inductoria are found, and there seems no valid reason why the general dimensions originally selected by Kronecker for his graduation should not be taken as standard. In a later paragraph (p. 55) observations will be cited which show that for quantitative work coils of this size are to be preferred to the smaller ones recommended by the Paris Congress.

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[8] See LEWANDOWSKI: Elektrodiagnostik und Elektrotherapie, Wien und Leipzig, 1887, p. 212; also HOORWEG: Die medicinische Elektrotechnik und ihre physikalischen Grundlagen, Leipzig, 1893, p. 128.

[9] KRONECKER: Arbeiten aus der physiologischen Anstalt zu Leipzig, 1871, p. 186.

TABLE II.

DETAILS OF CONSTRUCTION OF THE INDUCTORIA USED IN THIS STUDY.

Coil.	Length of secondary.	Turns in secondary.	Resistance of secondary.	Remarks.
	cm.			
A	12.5	10,000	850	Kronecker graduation
B	13.0	10,350	1400	Kronecker graduation
G	13.0	10,260	770	Kronecker graduation
E	6.5	5,000	300	Porter inductorium
H	9.3	6,000	450	
N	9.3	8,000	600	

Assuming as the standard, then, an inductorium having coils about 13 cm. long and having in the secondary approximately 10,000 turns of wire, we may inquire how widely it is possible for an inductorium to vary from this standard without introducing a significant error. For answering this question I have made observations with six inductoria, three of which are of "standard" construction and provided with Kronecker graduations, the other three selected so as to give increasing degrees of divergence from the standard. Details of the construction of the six inductoria are set down in Table II.

The results of my numerous experiments with these inductoria may be summarized as follows: The three standard coils, *A*, *B*, and *G*, give corresponding values of *Z* for equal stimuli whatever the secondary resistance. In comparing them, therefore, the factor of inductorium construction does not enter.

In thirteen experiments in which coil *N* was compared with coil *B* the secondary resistances ranged between 2850 ohms and 25,000 ohms; the average percentage variation of Z_N from Z_B was six per cent;[10] the greatest variation was 11.6 per cent. Z_N was greater than Z_B four times and Z_B greater than Z_N nine times. The small average percentage difference between the two coils, a difference only slightly greater than the probable experimental error, coupled with the fact that not all the variations are in the same direction, seems to me to show that in coils differing no more than these the influence of inductorium construction as a special factor can be disregarded without serious error.

TABLE III.

ILLUSTRATING THE INCREASING DIVERGENCE OF Z_E FROM Z_B WITH INCREASING SECONDARY RESISTANCE.

Resistance in sec. circuit.	Z_B.	Z_E.	Percentage variation of Z_B from Z_E.
900 ohms	15.75	16.05	1.9
1,600 ohms	17.9	19.8	10.6
2,700 ohms	18.7	26.8	43.0
8,600 ohms	24.1	46.0	91.0
20,600 ohms	49.0	135.0	175.0

An important effect of inductorium construction becomes manifest when coils *B* and *H* are compared. The average difference between Z_B and Z_H in twelve experiments was 16 per cent; in four of the twelve cases, moreover, the difference exceeded 24 per cent. Analyzing the series of experiments with reference to the secondary resistances, one finds that the high average difference is due to large differences in the experiments with high secondary resistance. Thus five experiments with secondary resistance above 10,000 ohms show an average difference between Z_B and Z_H of 25.7 per cent, while seven experiments with secondary resistances below 10,000 ohms show an average difference of only 4.5 per cent. In all five experiments with high secondary resistance Z_H was greater than Z_B. In the seven experiments with low resistance Z_H was larger than Z_B three times, smaller than Z_B twice, and equal to it twice.

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[10] For convenience of expression a subscript is placed after the value of *Z* to indicate with which coil the value was obained.

This series of experiments shows that in inductoria differing even so widely as do coils *B* and *H*, inductorium construction is unimportant so long as secondary resistance is kept low. It is, however, of great importance whenever the secondary resistance is high.

The final series of comparisons was between coil *B* and the Porter inductorium, coil *E*. This yielded results of the same sort as the preceding comparison but more marked. Only with very low secondary resistances were Z_B and Z_E equal, and Z_E becomes relatively more and more in excess of Z_B as the secondary resistance is increased. Table III illustrates this relationship very clearly

This last experiment brings out very clearly the objection to the standard set by the Paris Congress of 1881 (p. 52). The Porter inductoria conform closely to that standard in all respects save that of diameter of the coils. As Table III shows the influence of secondary resistance upon stimulating value is very much more marked in the small inductorium than in the larger one, so that while there are many sorts of experiments in which the investigator using a large coil is justified in disregarding secondary resistance, to do so would nearly always be unsafe if a small coil were being employed.

SUMMARY.

The conclusions to be drawn from the observations published in this paper are:

1. Determinations of the *specific* value of a faradic stimulus can be made accurately with any good inductorium of the sort found in physiological laboratories.

2. In making such determinations it is observed that the factor *A* in the expression for the specific stimulus $\beta = \frac{Z\,A}{A + R}$ varies with differences in inductorium construction. This factor is not, therefore, as formerly assumed, dependent solely on kathode surface, but in part as well on the construction of the inductorium.

3. The necessity for taking account of inductorium construction as a function of *A* can be avoided by selecting for quantitative use only inductoria conforming reasonably to a chosen standard; it is suggested that the dimensions selected by Kronecker in his graduation be taken as such standard.

ON THE PATHWAYS FOR THE BULBAR RESPIRATORY IMPULSES IN THE SPINAL CORD.

BY J. DEASON AND L. G. ROBB.

[*From the Hull Physiological Laboratory, University of Chicago.*]

THE experiments summarized in this report were carried out at the suggestion of Professor Carlson with the view of testing out a possible explanation of the Porter experiment on the spinal respiratory pathways.[1]

On repeating the well-known Porter experiment with a group of students in advanced physiology of the central nervous system, Professor Carlson found that after hemisection of the cervical cord and section of the opposite phrenic nerve in young kittens the respiratory impulses do not cross the median line in the cord if the animal is in a state of depression at the time of section of the phrenic. In such an animal, however, dyspnœa induces crossing of the impulses. Dyspnœa also leads to crossing of the impulses without previous section of the opposite phrenic nerve. This would seem to show that the crossed pathways for the respiratory impulses are normally open, and that their use depends upon the relative intensity of the impulses discharged from the bulbar centre.

The crossing of the respiratory impulses in the Porter experiment may be: I. A case of opening up of new reflex paths. The chief objections to this explanation are that the crossing takes place practically instantaneously on section of the opposite phrenic; and that dyspnœa induces the crossing, without the section of the opposite phrenic.

II. The section of the phrenic may raise the excitability of the phrenic and the bulbar respiratory centres, and thus increase the intensity of the respiratory impulses. In this case the Porter experiment becomes simply a special case of the spread of the reflex (or automatic) responses *pari passu* with the increased intensity of the stimulus (or the nervous impulses).

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[1] PORTER: Journal of physiology, 1895, xvii, p. 455.

The second hypothesis demands that the phrenic nerves contain afferent fibres, whose stimulation increases the intensity of the respiratory impulses; and that the crossing should be induced by the stimulation of any sensory nerve that causes an increase in the intensity of the bulbar respiratory discharges.

METHOD.

Kittens and dogs were used in these experiments. The kittens were chosen because of the rapidity with which they recover from shock. The movements of the diaphragm were observed by clipping the hair from the lower part of the thorax and abdomen, and noting external movements; by making an incision in the linea alba and applying digital palpation, and by direct observation of the diaphragm, both intact and divided into lateral halves by an incision from the xiphoid process to the spine.

The respiratory tracings were taken by connecting a tambour with the side arm of a *T* tube, which was placed between the ether bottle and the trachial cannula. In some of the experiments simultaneous blood pressure tracings were taken from the carotid artery.

All work was done with the animal under complete ether anæsthesia, care being taken to make this light and uniform.

RESULTS.

Confirmation of Porter's Experiment. In all of our experiments it was found that after hemisection of the cord in the upper cervical region (2d or 3d segments) the diaphragm on the side of hemisection was paralyzed, and provided the animals were in good condition, section of the opposite phrenic nerve immediately started the respiratory rhythm in the diaphragm on the side of the hemisection, followed by a permanent paralysis of the diaphragm on the side of the section nerve.

Porter assumes that "the section of the phrenic nerve interrupts the ordinary respiratory pathway on the same side, and a greater portion, perhaps the whole descending impulse of that side passes through the crossed dendrites to the phrenic cells of the opposite side." Unless the passage of of the bulbar respiratory impulses from their spinal conduction paths to the cells of origin of the phrenic motor fibres depends in some way on the normal impulses of afferent fibres in the phrenic; or unless the mere section of the phrenic motor fibres by antidromic impulses immediately blocks the passage from the spinal conduction paths to the phrenic cells, there is no reason for assuming such an "overflow" of the impulses to the opposite side.

The crossing of the bulbar respiratory impulses at the level of the phrenic nuclei after hemisection of the spinal cord can be induced by traction on the phrenic nerve of the intact side of the cord. The traction need not be great enough to block the impulses in the nerve. In fact, if it is properly adjusted, a uniform respiratory rhythm is induced in both sides of the diaphragm. Two typical protocols are submitted for illustration.

Experiment 33.— Young cat in good condition. Ether.

10.15 A.M. Animal anæsthetized.

10.26 A.M. Diaphragm exposed.

10.35 A.M. Left phrenic isolated.

10.43 A.M. Diaphragm divided from xiphoid process to spine, and artificial respiration started, both sides of diaphragm contracting evenly.

10.58 A.M. Cord hemisected in 2d cervical segment, right side. Very little hemorrhage. Right half of diaphragm paralyzed.

11.04 A.M. Left phrenic stretched until right diaphragm contracted.

11.05 A.M. Both sides of diaphragm contracting evenly. Inspected at intervals of from three to five minutes until 11.51.

11.51 A.M. Both sides of diaphragm contracting evenly. Animal killed. Post-mortem showed complete hemisection of the spinal cord.

Experiment 38.— Adult cat. Ether.

4.20 P.M. Abdomen opened. Diaphragm exposed.

4.26 P.M. Cervical cord exposed.

4.28 P.M. Left hemisection. Left side of diaphragm paralyzed. No hemorrhage.

4.36 P.M. Artificial respiration started and diaphragm sectioned from ensiform process to spine.

4.41 P.M. Right phrenic isolated just above diaphragm. Trauma of isolation caused crossing of impulse. Diaphragm examined at intervals of three to five minutes and both sides were contracting equally at 5.40.

5.41 P.M. Animal killed.

Post-mortem showed complete hemisection of the spinal cord.

Stimulation of the central end of the sectioned phrenic nerve causes an increase in both rate and amplitude of the respiratory movements, preceded in some cases by a slight inhibition or irregularity. The effects on the blood pressure are similar to those following the stimulation of the central end of any sensory nerve. Mechanical stimuli, *i. e.*, ligation and pressure on the phrenic nerves, give the same results as electrical stimulation.

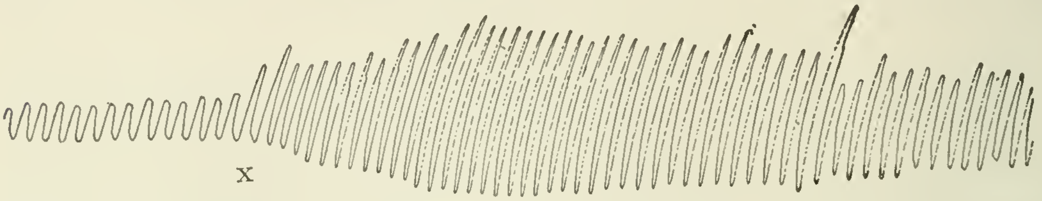

FIGURE 1. — One half the original size. Cat. Hemisection of the spinal cord in third cervical segment. Tracing of the respiratory movements via the tracheal cannula. *X* = ligation of the phrenic nerve on the opposite side of the hemisection. Increased activity of the respiratory centre.

Typical tracings illustrating this phenomenon are reproduced in Figs. 1–5. Weak stimuli tend to augment the intensity of the respiratory discharges, while strong stimuli augment both the rate and intensity of the impulses. The effects may persist for two to ten minutes after the cessation of the stimulation. The fact that the afferent phrenic fibres are strongly stimulated by the mere sectioning of the phrenic seems to show that this is a factor in the immediate crossing of the respiratory impulses in the Porter experiment. But, as the

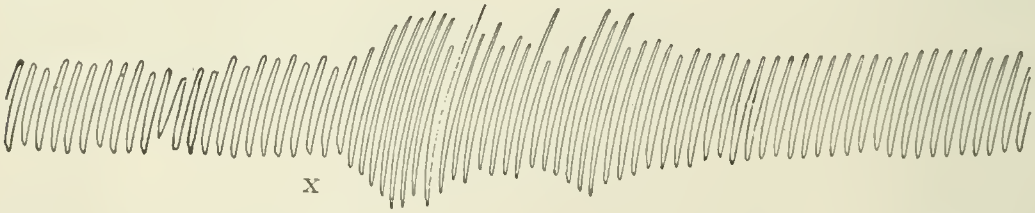

FIGURE 2. — One half the original size. Cat. Tracing of respiratory movements via the tracheal cannula. *X* = section of the left phrenic nerve after previous hemisection of the right side of the spinal cord between the second and third cervical vertebræ. Increased activity of the respiratory centre.

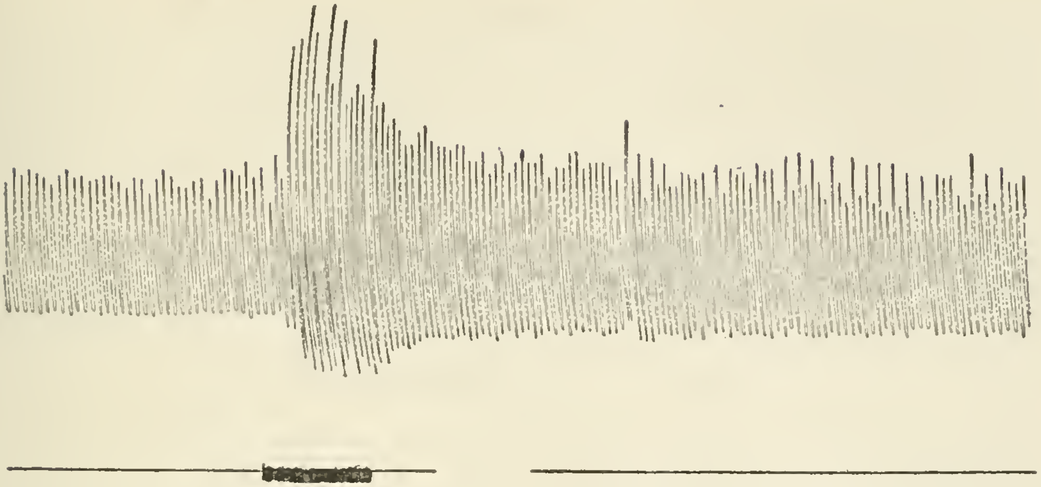

FIGURE 3. — Four fifths the original size. Cat. Tracing on stimulation of the central end of the left phrenic nerve, right phrenic nerve intact. Increased activity of the respiratory centre.

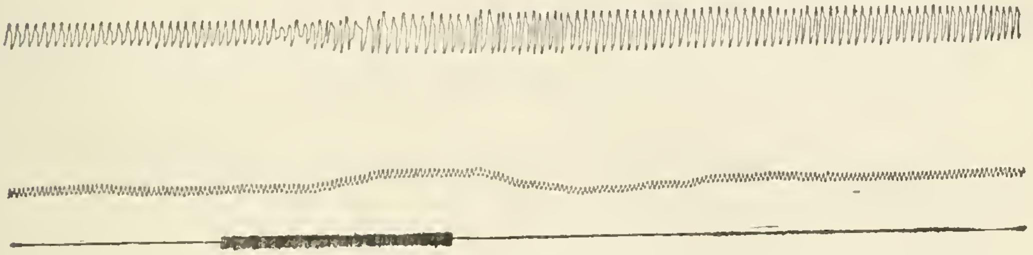

FIGURE 4. — Two fifths the original size. Cat. Tracings of the carotid pressure and the respiratory movements. Both phrenic nerves sectioned. Stimulation of central end of left phrenic nerve. Increased activity of the respiratory centre.

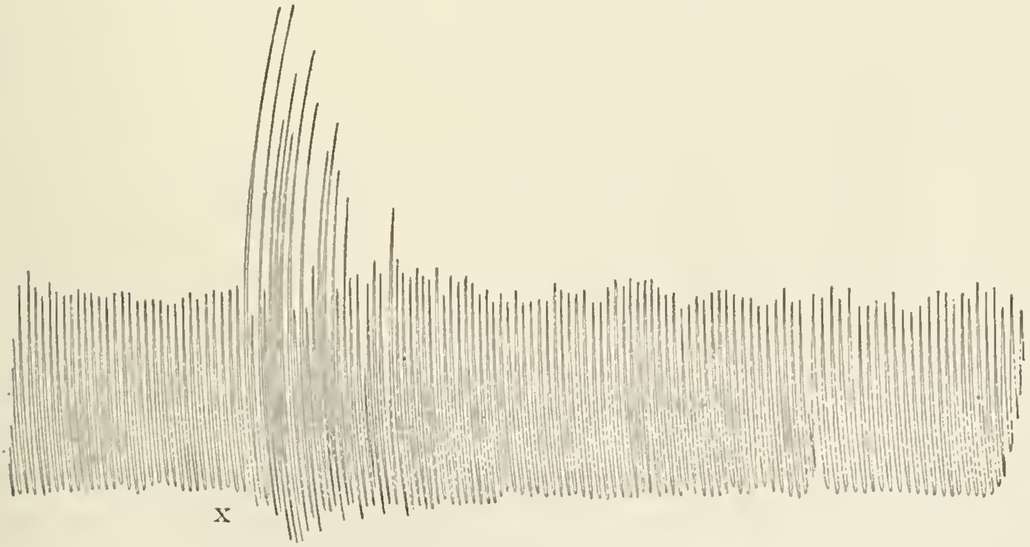

FIGURE 5. — Eight ninths the original size. Cat. Respiratory tracing via the tracheal cannula. *X* = light traction on central end of the left phrenic nerve. Increased activity of the respiratory centre.

crossing persists, according to Porter's observations, after such stimulation of the afferent fibres due to the section of the nerve has presumably ceased, other factors must also be involved, possibly an increased excitability in the phrenic nuclei, due to injury currents and products of degeneration.

Stimulation of the sciatic nerve induces crossing of the respiratory impulses and an equal contraction of each lateral half of the diaphragm, which may continue for ten minutes after the cessation of the stimulation.

One typical experiment may be cited for illustration.

Experiment 26.— Kitten. Ether.

3.50 P.M. Cervical cord exposed.

3.54 P.M. Abdomen opened.

3.56 P.M. Diaphragm sectioned from xiphoid process to spine. Artificial respiration started.

4.05 P.M. Cervical cord hemisected, right side; right half of diaphragm paralyzed.

4.15 P.M. Electrical stimulation, central end of right sciatic. Both sides of diaphragm contracting equally and synchronously.

4.25 P.M. Right half of diaphragm ceased contracting.

4.29 P.M. Electrical stimulation central end of right sciatic. Respiratory rhythm of entire diaphragm.

4.31 P.M. Right side of diaphragm ceased contracting.

4.34 P.M. Electrical stimulus central end of right sciatic. Both sides of diaphragm contracting.

4.36 P.M. Right side of diaphragm ceased contracting.

4.40 P.M. Artificial respiration stopped for twelve seconds. Right side of diaphragm contracting.

4.40.12 P.M. Artificial respiration started.

4.40.27 P.M. Right diaphragm ceased contracting.

4.44 P.M. Left phrenic sectioned. Left side of diaphragm paralyzed. Right diaphragm contracting.

4.50 P.M. Animal killed.

SUMMARY AND CONCLUSIONS.

1. The phrenic nerves contain afferent fibres, the stimulation of which augments the intensity and the rate of the bulbar respiratory impulses. The effects of stimulation of these fibres on the respiratory centre outlasts for varying lengths of time the period of stimulation.

2. After hemisection of the spinal cord between the medulla and the phrenic nuclei and consequent paralysis of the diaphragm on the hemisected side, the bulbar respiratory impulses will cross from the intact to the hemisected side of the spinal cord on stimulation of the sciatic nerve; on traction or mechanical stimulation of the phrenic nerve on the intact side of the cord; in dyspnœa; as well as on section of the phrenic nerve on the intact side. These various conditions induce increased intensity of the bulbar respiratory impulses. The immediate crossing of the bulbar respiratory impulses on section of the phrenic nerve after previous hemisection of the opposite side of the cervical cord seems therefore to be a case of the spread of reflex (or automatic) responses *pari passu* with the increased intensity of the nervous impulses. But since in the Porter experiment the crossing of the impulses appears to be permanent, additional factors are probably involved.

EMOTIONAL STIMULATION OF ADRENAL SECRETION.

By W. B. CANNON and D. de la PAZ.

[*From the Laboratory of Physiology in the Harvard Medical School.*]

IN 1891 Jacobi described nerves branching from the splanchnics and supplying the adrenal glands.[1] Six years later Biedl found that these nerves conveyed vaso-dilator impulses to the glands, and suggested that they probably conveyed also secretory impulses.[2] Evidence for this suggestion was presented the following year by Dreyer, who demonstrated that electrical stimulation of the splanchnics below the diaphragm produced in the adrenal blood an increased amount of substance raising arterial blood pressure and that this result was independent of accompanying vascular changes in the glands.[3] The work of Dreyer has been confirmed by Tscheboksaroff[4] and by Asher.[5] The conclusion is justified, therefore, that adrenal secretion is under control of the thoracico-lumbar autonomic (sympathetic) system.

The phenomena of a major emotional exhibition in an animal indicate the dominance of sympathetic impulses. When, for example, a cat becomes frightened, the pupils dilate, the stomach and intestines are inhibited, the heart beats rapidly, the hairs of the back and tail stand erect — all signs of nervous discharges along sympathetic paths. Do not the adrenal glands share in this widespread subjugation of the viscera to sympathetic control?

In order to test this suggestion the natural enmity between two laboratory animals, dog and cat, was utilized. The cat, fastened to a comfortable holder, was placed near a barking dog. Some cats showed almost no signs of fear; others, with scarcely a movement of defence, made the typical picture of fright. In favorable cases the excitement was continued for five or ten minutes, and, in a few instances, longer. Samples of blood were taken within a few minutes before and after the period.

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[1] Jacobi: Archiv für experimentelle Pathologie und Pharmakologie, 1891, xxix, p. 185.

[2] Biedl: Archiv für die gesammte Physiologie, 1897, lxvii, pp. 456, 481.

[3] Dreyer: This journal, 1898–1899, ii, p. 219.

[4] Tscheboksaroff: Archiv für die gesammte Physiologie, 1910, cxxxvii, p. 103.

[5] Asher: Zentralblatt für Physiologie, 1910, xxiv, p. 927.

In the first experiments the blood was obtained by cardiac puncture. An attempt was made to test for the presence of adrenal secretion by the frog-eye method suggested by Meltzer[6] and Ehrmann.[7] The changes in size of the pupil, which were recorded by means of a camera lucida, did not give sufficiently striking results to permit drawing conclusions. Strips of beef artery, employed by Meyer,[8] though highly sensitive, did not seem serviceable because of Schlayer's discovery that the method is less efficient when used with foreign blood.[9] Structures natural to the blood to be tested were the uterus and strips of longitudinal muscle from the cat's intestine. Magnus showed, in 1905,[10] that longitudinal intestinal muscle, contracting rhythmically, is characteristically inhibited by suprarenin, 1:20,000,000. Though this reaction has not hitherto been utilized as a biological signal for adrenal secretion, it possesses noteworthy advantages over the other methods. The strip is found in all animals, and not in only half of them, as is the uterus; it is ready for the test within a few minutes, instead of the several hours said to be required for the best use of the uterus method;[11] it need not be stretched in order to be ready to contract, as with the arterial strip; and it responds by relaxing. The last characteristic is especially important, since other substances in blood than adrenal secretion, as, for example, carbon dioxide, will cause smooth muscle to contract, whereas known substances evoking relaxation of smooth muscle are few and unusual in blood.[12]

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[6] MELTZER and C. M. AUER: This journal, 1904, xi, p. 449.

[7] EHRMANN: Archiv für experimentelle Pathologie und Pharmakologie, 1905, liii, p. 97.

[8] MEYER: Zeitschrift für Biologie, 1906, xlviii, p. 352.

[9] SCHLAYER: Deutsche medizinische Wochenschrift, 1907, xxxiii, p. 1898.

[10] MAGNUS: Archiv für die gesammte Physiologie, 1905, cviii, p. 48.

[11] FRAENKEL: Archiv für experimentelle Pathologie und Pharmakologie, 1909, lx, p. 399.

[12] GRÜTZNER: Ergebnisse der Physiologie, 1904, iii^2, p. 66; MAGNUS: *Loc. cit.*, p. 69.

The strip of longitudinal intestinal muscle, attached to a writing lever, was suspended between *serres fines* in a cylindrical chamber, 8 mm. in diameter and 5 cm. deep, through which oxygen passed. When not exposed to blood, the strip was immersed in Ringer's solution at body temperature. The chamber, the stock of Ringer's solution, and the blood samples were all surrounded by a large volume of water kept at approximately 37° C.

Although blood obtained by cardiac puncture gave in some cases characteristic results by this method, blood from the inferior vena cava was so much more regular and differential in its effects that it alone was finally used. To obtain blood from the inferior cava above the opening of the adrenal vessels, the skin over the femoral in the groin was made anæsthetic with ethyl chloride; the vein was bared, cleaned, tied, and opened; and a small flexible catheter (2.4 mm. diameter), coated with vaseline inside and out, was introduced through the iliac into the cava to near the level of the sternal notch. A ligature around the catheter at the point where it disappeared into the vessel permitted later reintroduction to the same extent. Since there was no sign of sensation when the catheter was slipped into the vein, it was possible to obtain "quiet blood," with only local anæsthesia. As soon as the blood (3 or 4 c.c.) was drawn, the catheter was removed and the vein tied. The blood was immediately defibrinated. After the animal had been frightened, the procedure was repeated, and thus the "excited blood" was secured. The two samples of blood, both treated in the same manner, and both kept at the same temperature as the contracting strip, were now tested.

The first effect of adding blood, whether quiet or excited, to the muscle strip was to send it into a strong contraction which might persist, sometimes with slight oscillations, for a minute or two (see Figs. 2 and 3). This contraction was not due to carbon dioxide, for removal of the blood gases and later restoration of oxygen did not alter the effect. Blood serum from centrifugated clot, and blood kept fluid by hirudin, produced equally the initial shortening.

After the initial shortening the strip, if in quiet blood, soon began to contract rhythmically and at the same time to lengthen more with each relaxation, until a fairly even base line appeared in the written record. At this stage the addition of fresh quiet blood usually had no effect, even though the strip were washed once with Ringer's solution before the second portion of the blood was added. In comparing the effects of quiet and excited blood on the contracting strip, the two samples were each added to the strip immediately after removing the Ringer's solution, or they were applied to the strip alternately, and the differences in effect then noted.

That blood from the adrenal veins causes the relaxation of intestinal muscle characteristic of adrenal extract is shown in Fig. 1. The strip was originally beating in blood which contained no demonstrable amount of adrenal secretion; that blood was replaced by blood from the adrenal veins, obtained after quick etherization. Relaxation occurred almost immediately. Then the beats were renewed in the former blood, and thereupon the strip was immersed in blood from the left renal vein, obtained from the same animal and under the same conditions with the adrenal blood. No relaxation occurred. This and other similar tests proved the reliability of the method.

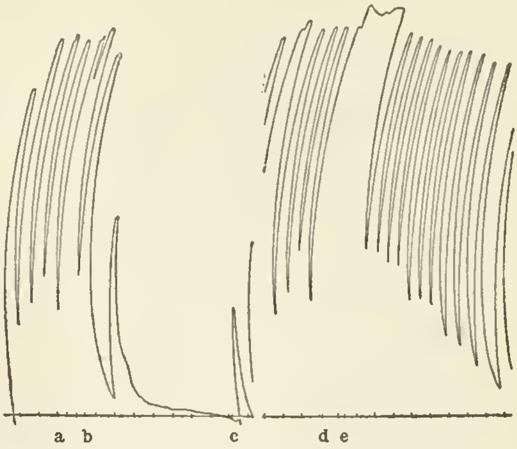

FIGURE 1. — Two fifths the original size. Intestinal strip beating in inactive blood which was removed at *a*. Blood from adrenal veins substituted at *b*, and removed at *c*. Contractions restored in inactive blood, removed at *d;* then blood from renal vein (same animal) added at *e*. In this and in the other tracings time is recorded in half minutes.

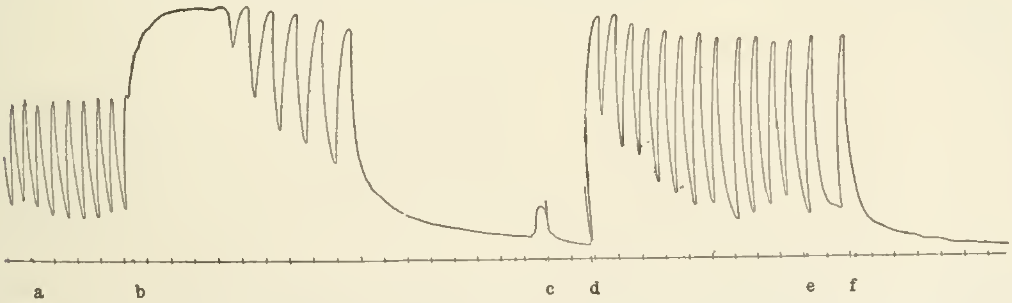

FIGURE 2. — About two fifths the original size. Alternate application of excited blood (at *b* and *f*) and quiet blood (at *d*), from the same animal, to an intestinal strip initially beating in Ringer's solution. See text for further description.

In no instance did blood from the quiet normal animal produce relaxation. On the other hand, blood from the animal after the emotional disturbance showed more or less promptly the typical relaxation. In Fig. 2 is presented the record of a strip which at first was beating regularly in Ringer's solution. At *a* the Ringer's solution was removed and at *b* excited blood was added; after a preliminary shortening the strip lengthened gradually into an inhibition. At *c* the excited blood was removed, and at *d* quiet blood was added in its place. The strip at once began fairly regular rhythmic beats. At *e* the quiet blood was removed, and at *f* the excited blood again applied. The strip lengthened almost immediately into an inhibited state. In this instance the animal was excited about fifteen minutes.

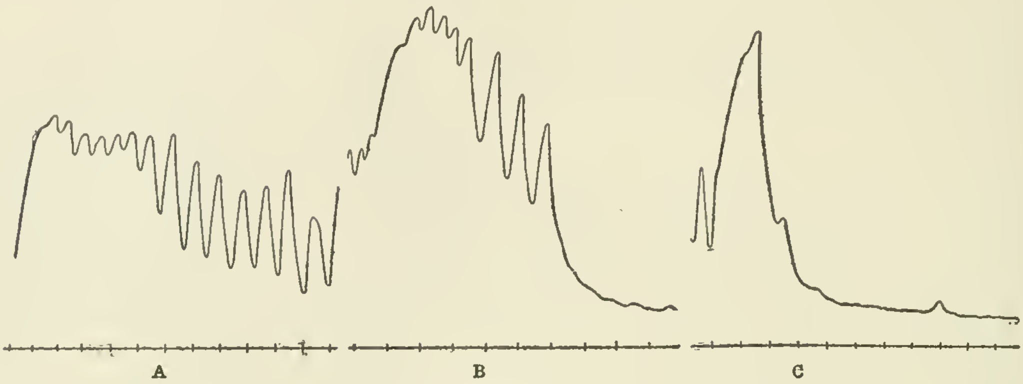

FIGURE 3. — About two thirds the original size. The effect of prolongation of excitement. *A*, the record in quiet serum; *B*, in defibrinated blood after eleven minutes, of excitement, and *C*, in serum after fifteen minutes of excitement.

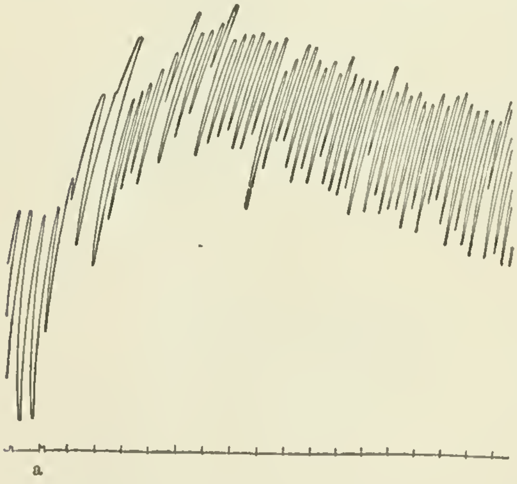

FIGURE 4. — One third the original size. The failure of blood to produce inhibition when excitement has occurred after removal of the adrenal glands. At *a*, blood added from inferior cava of excited animal previously deprived of adrenal glands. The strip later proved sensitive to adrenalin in blood in the ratio 1 : 1,000,000.

The increase of effect with prolongation of the emotional period is shown in Fig. 3. *A* is the record in quiet serum; *B*, the record in defibrinated blood after eleven minutes of excitement; and *C*, the record in serum after fifteen minutes of excitement. In other instances the effect was manifested merely by a lowering of the tonus of the strip and a notable slowing of the beats, without, however, a total cessation.

The view that the effects of excited blood are due to an increased content of adrenal secretion is justified for several reasons. (1) The effect has been obtained in blood from the inferior vena cava near the liver, when blood from the femoral vein, taken at the same time, produced no inhibition. Since the femoral blood typifies the cava blood below the kidneys, the conclusion is warranted that the effect is not produced below the entrance of the kidney veins. That blood from the kidney veins does not cause the relaxation is shown in Fig. 1. (2) If the blood vessels of the adrenal glands are first carefully tied and the glands then removed, excitement four or five hours later does not alter the blood so that the typical effect occurs (see Fig. 4), although the animal shows all the characteristic indications of sympathetic stimulation. (3) Varying amounts of adrenalin added to blood which has not produced relaxation of the strip, evoke all the degrees of relaxation that have been observed in excited blood; Fig. 5 shows the

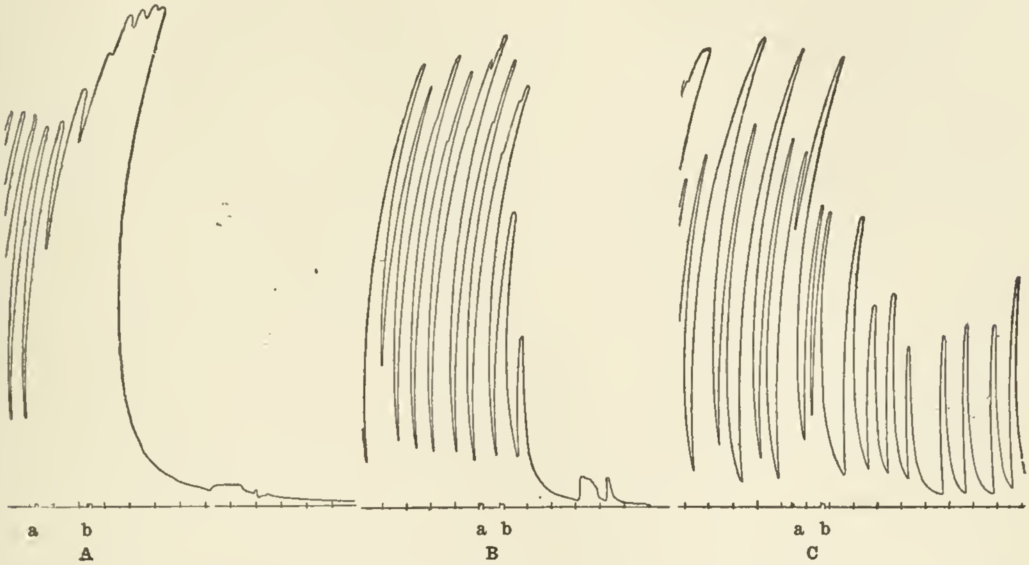

FIGURE 5. — About one half the original size. Effect of adding adrenalin 1 : 1,000,000 (*A*), 1 : 2,000,000 (*B*), and 1 : 3,000,000 (*C*), to formerly inactive blood. In each case *a* marks the moment when the quiet blood was removed, and *b*, the time when the blood with adrenalin was added.

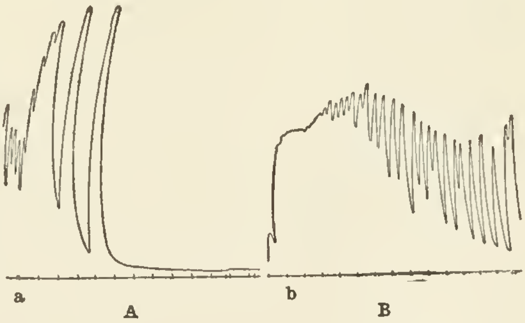

FIGURE 6. — One third the original size. The effect of bubbling oxygen through active blood. *A*, relaxation after active blood applied at *a*; *B*, failure of relaxation when the same blood, oxygenated three hours, was applied to a fresh strip at *b*.

effect of adding adrenalin, 1 : 1,000,000 (*A*), 1 : 2,000,000 (*B*), and 1 : 3,000,000 (*C*), to previously inactive blood. (4) Excited blood which produces inhibition loses that power on standing in the cold for twenty-four hours or on being kept warm and agitated with bubbling oxygen for only two or three hours. This last effect is illustrated in Fig. 6; the power to inhibit possessed when record *A* was written was lost in three hours in the presence of bubbling oxygen. Embden and v. Fürth have reported that 0.1 gm. of suprarenin chloride disappears almost completely in two hours if added to 200 c.c. of defibrinated beef blood, and the mixture constantly aerated at body temperature.[13] All these considerations, taken with the evidence that sympathetic impulses increase the secretion of the adrenal glands, and that during such emotional excitement as was employed in these experiments, signs of sympathetic discharges were observable from the eye of the animal to the tip of its tail, prove that the characteristic effect of adrenal extract on the intestinal strips was due to secretion of the adrenal glands.

After excitement lasting ten minutes or more the effect was demonstrable not only in heart blood but also in blood from the femoral vein. As is well known, adrenal secretion itself is capable of working the effects evoked by sympathetic stimulation. Elliott reported that injected adrenalin discharges the adrenal glands so that the medullary cells stain less deeply with chromate salts, and yield no active extract.[14] It is conceivable, therefore, that some of the adrenal secretion set free by nervous stimulation returns to the glands in the blood stream, and, within limits, stimulates them to further activity. Thus the more marked effect as time passes (see Fig. 3) may be due not only to further excitement, but in part to an autogenous continuance of adrenal secretion. Thus also the persistence of the emotional state after the exciting object has disappeared can be explained. Indeed it was the lasting effect of excitement on digestive processes which suggested this investigation.

[13] Embden and v. Fürth: Hofmeister's Beiträge zur chemischen Physiologie und Pathologie, 1904, iv, p. 423.

[14] Elliott: Journal of physiology, 1905, xxxii, p. 427.

PROTEIN METABOLISM IN PHLORIZIN DIABETES.

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THE relation of kreatin to kreatinin in protein metabolism has not as yet been satisfactorily explained, and late observations on the excretion of these substances by a number of investigators seem to point to a closer relationship than was at first suggested by Folin.

On the other hand, the appearance of kreatin in the urine in cases of eclampsia,[1] typhoid fever,[2] and pneumonia[3] which has been pointed out by one of us with the workers in this laboratory indicates that kreatin has a significance quite apart from kreatinin in showing unusual disturbances of metabolism.

That the appearance of kreatin in the urine is not always associated with loss of protein is shown most clearly by the experiments of Schwarz,[4] made in this laboratory. In investigations of the metabolism of rachitic children, Schwarz has shown that the urine contains kreatin, although they were in distinct positive nitrogen balance and were increasing in weight.

We have also shown that carbohydrates and proteins much below the amount necessary to compensate for the caloric loss of an animal are able to prevent the appearance of kreatin in the urine, or to cause a cessation of its presence, once it has been established by fasting. A full report of this work will appear elsewhere.

In a recent paper Cathcart and Taylor[5] have investigated the relation between the nitrogen loss to the body and the appearance of kreatin in the urine. They used for this purpose the method of rendering animals glycosuric with phlorizin. As a result of these experiments, it is concluded that a sufficient amount of carbohydrate in the food will prevent the appearance of kreatin in the urine, and that its presence there is conditioned by nitrogen loss to the body. They also say that there is no connection between acidosis and the disturbance of metabolism leading to acidosis.

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[1] Ewing and Wolf: American journal of obstetrics, 1906, cxxxi, p. 751.

[2] Ewing and Wolf: Archives of internal medicine, 1909, iv, p. 331.

[3] Wolf and Lambert: Archives of internal medicine, 1910, v, p. 406.

[4] Schwarz: Jahrbuch für Kinderheilkunde, 1910, lxxii, p. 549.

[5] Cathcart and Taylor: Journal of physiology, 1910, xli, p. 276.

TABLE I.

Day.	Treatment of dog.	Wt. of dog. 8580 gm. gain = + loss = −	Urine volume. c.c.	Nitrogen	
			Spec. gr.	Total gm.	Ammon. gm. *% T−N.*
1	Food = 4.5 gm. N. — 55 calor. pr. kilo.	+ 20	110 *1038*	4.479	0.147 *3.29*
2	Food = 4.5 gm. N. — 55 calor. pr. kilo.	— 20	120 *1037*	4.530	0.176 *3.88*
3	Food = 4.5 gm. N. — 55 calor. pr. kilo.	+ 20	160 *1030*	4.895	0.185 *3.79*
4	No food	— 220	55 *1040*	2.425	0.072 *2.96*
5	No food	— 200	64 *1044*	2.374	0.077 *3.25*
6	No food	— 200	54 *1048*	2.242	0.077 *3.45*
7	No food	— 180	50 *1049*	2.303	0.094 *4.08*
8	No food	— 80	47 *1044*	2.390	0.114 *4.76*
9	Eats 85 gm. olive oil = 100 calor. pr. kilo.	— 120	50 *1053*	2.341	0.121 *5.19*
10	No food	— 80	55 *1039*	2.451	0.135 *5.54*
11	No food	— 80	37 *1056*	2.294	0.096 *4.19*
12	Eats 83 gm. olive oil — 6 gm. phlorizin injected	— 140	280 *1060*	7.290	0.428 *5.88*
13	No food — 6 gm. phlorizin injected	— 400	500 *1055*	10.900	0.814 *7.64*
14	No food 6 gm. phlorizin injected	— 380	545 *1053*	11.830	0.830 *7.02*
15	No food 6 gm. phlorizin injected	— 140	440 *1065*	12.200	0.725 *5.94*
16	5 gm. glutaric acid and 6 gm. phlorizin injected	— 360	700 *1033*	8.282	0.458 *5.53*

The glycosuria produced by Cathcart and Taylor was not intense, and as the amount of carbohydrate administered is not exactly given, it is not possible to estimate the glucose-nitrogen ratio indicating any formation of glucose from body protein.

The present investigation was undertaken to throw light on the behavior of kreatin in starvation. We wished at the same time to obtain information regarding any connection between acidosis and the appearance of kreatin.

TABLE I.

in urine.			Total sulphur. gm.	Glucose. gm.	Acetone.		β Oxy-butyric acid. gm.
Kr'inin + kr'n. gm.	Kreatinin. gm.	Kreatin. gm.			Pre-formed. gm.	Aceto-acetic acid. gm.	
% T−N.	*%·T−N.*	*% T−N.*	*1−100 N: S.*	*1:1 N:Glucose.*			
....	0.1125 *2.51*	0	0.2538 *5.67*	0	0.0036	0	0.0183
....	0.1140 *2.52*	0	0.2382 *5.26*	0	0.0050	0	0.0246
....	0.1110 *2.27*	0	0.2580 *5.27*	0	0.0048	0	0.0278
0.0930 *3.83*	0.0870 *3.59*	0.0060 *0.24*	0.1582 *6.53*	0	0.0040	0	0.0192
0.0970 *4.09*	0.0806 *3.40*	0.0164 *0.69*	0.1500 *6.32*	0	0.0032	0	0.0230
0.0930 *4.15*	0.0720 *3.21*	0.0210 *0.94*	0.1330 *5.93*	0	0.0032	0	0.0165
0.0970 *4.21*	0.0790 *3.43*	0.0180 *0.78*	0.1340 *5.82*	0	0.0056	0	0.0157
0.1070 *4.48*	0.0830 *3.47*	0.0240 *1.01*	0.1412 *5.89*	0	0.0028	0	0.0193
0.1050 *4.48*	0.0810 *3.46*	0.0240 *1.02*	0.1374 *5.87*	0	0.0048	0	0.0265
0.1070 *4.37*	0.0778 *3.18*	0.0292 *1.19*	0.1464 *5.97*	0	0.0048	0	0.0186
0.1025 *4.47*	0.0825 *3.60*	0.0200 *0.87*	0.1441 *6.28*	0	0.0060	0	0.0158
0.1520 *2.08*	0.1100 *1.51*	0.0420 *0.57*	0.4592 *6.30*	35.20 *4.83*	0.0530	0.1550	0.8664
0.2167 *1.99*	0.0924 *0.84*	0.1243 *1.15*	0.6435 *5.90*	35.20 *3.23*	0.1144	0.5544	3.009
0.2336 *1.97*	0.1072 *0.90*	0.1264 *1.07*	0.7056 *5.97*	37.60 *3.18*	0.1568	0.2672	2.113
0.2496 *2.05*	0.1024 *0.84*	0.1472 *1.21*	0.7840 *6.42*	39.12 *3.21*	0.0768	0.1632	1.260
0.3120 *3.77*	0.1000 *1.21*	0.2120 *2.56*	0.6180 *7.46*	29.00 *3.50*	0.0400	0.1800	1.253

Reasoning *a priori*, there should be little connection between these two processes, for while kreatin appears in the urine of fasting dogs, acidosis does not appear in this animal until the fasting animal has been rendered glycosuric with phlorizin (v. Mering,[6] Waldvogel,[7] Baer [8]).

If kreatin be connected in any way with a disturbed carbohydrate metabolism, or on the other hand be incident to certain types of tissue katabolism only seen in starvation after the reserve store of glycogen has been used up, as fasting experiments suggest; then animals rendered completely diabetic with phlorizin should afford information regarding any connection between the formation of sugar from protein and the excretion of kreatin.

TABLE II.

Day.	Treatment of dog.	Weight of dog. 12000 gm. gain + loss −	Volume urine. c.c. *Spec. gr.*	Nitrogen in		
				Total gm.	Ammon. gm. *% T−N.*	Kr'tinin and kr'n. gm. *% T−N.*
1	No food	− 260	112 *1025*	2.340	0.232 *9.91*	....
2	No food	− 240	80 *1029*	2.044	0.150 *7.34*	....
3	No food	− 140	85 *1027*	1.872	0.121 *6.46*	....
4	No food	− 140	72 *1029*	2.100	0.152 *7.26*	0.1120 *5.34*
5	No food. 6 gm. phlorizin injected	− 220	350 *1041*	5.234	0.192 *3.67*	0.1720 *3.29*
6	No food. 6 gm. phlorizin injected	− 200	720 *1033*	9.000	0.464 *5.16*	0.3250 *3.61*
7	No food. 6 gm. phlorizin injected	− 220	740 *1032*	8.954	0.535 *5.98*	0.3267 *3.65*
8	No food. 6 gm. phlorizin injected	− 400	760 *1030*	8.899	0.468 *5.26*	0.3717 *4.18*
9	No food. 6 gm. phlorizin 5 gm. glutaric acid	− 500	....	5.575	0.310 *5.56*	0.1600 *2.87*

It is known that by the proper administration of phlorizin to fasting animals a constant ratio of glucose to total nitrogen may be obtained. If the poison be effective, an amount of glucose will be excreted which shows that all the sugar produced from protein is excreted without being utilized.

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[6] v. MERING: Zeitschrift für klinische Medizin, 1889, xvi, p. 442.

[7] WALDVOGEL: Zeitschrift für klinische Medizin, 1899, xxxviii, p. 506.

[8] BAER: Archiv für experimentelle Pathologie und Pharmakologie, 1904, li, p. 271.

As Cathcart and Taylor have made use of a temporary and non-fatal poisoning with phlorizin in fed animals, we have changed the experiment and made fasting dogs completely diabetic, and continued the action of the poison for some days. Our object was to influence the fasting protein metabolism as completely as possible.

TABLE II.

urine.		Total sulphur. gm.	Glucose. gm.	Acetone.			Water given. c.c.	Temp.
Kr'tinin. gm.	Kreatin. gm.			Pre-formed. gm.	Aceto-acetic acid. gm.	β Oxy-butyric acid. gm.		
% T−N.	*% T−N.*	1:100 *N:S.*	1:1 *N:Gl'se.*					
0.1000 *4.27*	0	0.1712 *7.32*	0	0.0080	0	0.0208		
0.0975 *4.77*	0	0.1470 *7.20*	0	0.0090	0	0.0260		38.0
0.0765 *4.09*	0	0.1128 *6.00*	0	0.0096	0	0.0171	135	38.3
0.0920 *4.38*	0.0200 *0.96*	0.1340 *6.38*	0	0.0072	0	0.0214	135	
0.1160 *2.22*	0.0560 *1.07*	0.3144 *6.00*	29.68 *5.67*	0.0400	0.0320	0.1610	300	38.6
0.1230 *1.37*	0.2020 *2.24*	0.5710 *6.34*	36.00 *4.00*	0.1000	0.1350	0.7830	700	38.4
0.1144 *1.28*	0.2120 *2.37*	0.6732 *7.52*	33.00 *3.70*	0.1804	0.2536	1.7720	550	38.4
0.1022 *1.15*	0.2695 *3.03*	0.6468 *7.27*	31.13 *3.52*	0.2332	0.2180	2.0580	600	39.0
0.0726 *1.30*	0.0874 *1.57*	0.4940 *8.86*	17.00 *3.04*	0.2200	0.0460	0.8320		35.8

The following experiments were performed on two animals. The phlorizin period was preceded in the one case by eight days' fasting and in the second by four days' fasting. The phlorizin used was Kahlbaum's. The drug was administered subcutaneously in 2.0 gram doses, dissolved in 25 c.c. of warm sodium carbonate solution. It was given three times daily, at 8.00 A. M., 4.00 P. M., and at 10.00 P. M., or midnight. The animals were catheterized twice a day, at 9.00 A. M. and 6.00 P. M.

The methods for the determination of ammonia, kreatinin, kreatin, acetone and acetoacetic acid were those of Folin. Shaffer's method was used for the determination of β oxybutyric acid. Sulphur was estimated by Benedict's method. The glucose was determined by Bang's method, after the urine had been decolorized with acid mercuric nitrate.

The results were controlled by the Allihn-Soxhlet method. The agreement between the two methods was perfectly satisfactory. It will be noted that the results for ammonia, kreatinin, and kreatin are expressed in terms of nitrogen.

It has been stated by Folin, himself, that the picric acid method for the determination of kreatinin and kreatin must be employed with caution when samples containing acetone compounds are employed.

TABLE III.

Character of urine.	Nitrogen %.		
	Kreatinin + kreatin.	Kreatinin.	Kreatin.
Urine, man	0.063	0.051	0.012
Urine, man, + 1% acetoacetic ester	0.063	0.055	0.008
Urine, dog, made up to 500 c.c. for kreatin in estimation	....	0.053	......
Urine, dog, made up to 1000 c.c. for kreatin in estimation	....	0.052	......
Urine, dog, 1 % acetoacetic ester dilution 500 c.c.	....	0.051	......
Urine, dog, 1 % acetoacetic ester dilution 1000 c.c.	....	0.050	......
Urine, dog, + 0.05 phlorizin	....	0.050	

We therefore endeavored to assure ourselves that the kreatinin and kreatin values obtained in these experiments were not complicated by the presence of these compounds.

In order to note the effect of the change of acetoacetic acid to acetone, two sets of determinations were made, separated by a period of nearly a month. The results were practically concordant.

Determinations of kreatinin + kreatin were made in urines which had been heated with acid while air was passed through to completely remove the acetone. As might be anticipated, these results were the same as with the simple treatment with heat and acid.

Finally, acetoacetic ester was added directly to urine which had previously been analyzed for kreatinin and kreatin. As will be noted in Table III, this compound does affect the kreatinin determinations, but not to an extent which would vitiate the experimental results which we have obtained.

One other possibility was present — that is, the effect of phlorizin itself on kreatinin determinations. As is well known, this substance is excreted by the kidney.

The ferric chloride reaction, which is obtained with phlorizin urines, is due in great part to this substance. It differs, however, from the typical Gerhardt reaction in persisting after the urine has been heated. Phlorizin also has the property of giving with picric acid and alkali a color which in concentrated solution is indistinguishable from that produced by kreatinin under the same conditions. Fortunately, the colored compound is highly dissociated on dilution, so that, after adding water, the red color, which would imitate kreatinin picrate, entirely disappears.

As these animals received 6.0 gm. of phlorizin in the twenty-four hours, and the urine was diluted for analysis to 1000 c.c., there could be no more than 0.060 gm. in the amount of urine taken for analysis.

This amount of phlorizin, when treated with picric acid and sodium hydroxide, in the quantities used for a kreatinin determination, failed to give a color which could be estimated with the colorimeter when the solution was diluted to 500 c.c.

We therefore conclude that neither acetoacetic acid nor phlorizin is responsible for the variation in kreatinin and kreatin which is observed after treating fasting animals with phlorizin.

The Results.

Animal 1. — The usual phenomena connected with fasting are observed in this animal. Kreatin does not appear until the third day of starvation, when 0.016 gm. of nitrogen are excreted in this form. The kreatinin output falls. This is, however, only absolute, for the ratio of kreatinin nitrogen to total nitrogen rises. The administration of fats in the form of olive oil has no effect in sparing fasting metabolism, or in decreasing the amount of kreatin.

With the rise in nitrogenous metabolism occasioned by the injection of 6 gm. of phlorizin, the amount of kreatinin increased, but its elimination does not in any sense keep pace with the total nitrogen excretion. For that reason the ratio of kreatinin nitrogen to total nitrogen falls. It is obvious, therefore, that the loss of body protein occasioned by this poison does not equally affect the processes leading to the excretion of kreatinin. This difference may lie in the breaking down of muscle tissue as distinguished from that form of protein breakdown which has been characterized by Noel Paton as " meat."

On the other hand, we find that both total nitrogen and kreatin nitrogen increase with equal steps. It would appear that whatever tissue or process was contributing to the excess of nitrogen in the urine was yielding an equal amount of kreatin. It is worthy of mention that during the period when the excess of glucose was being swept out of the body the ratio of kreatin nitrogen to total nitrogen was low. In the preceding period, when no sugar was excreted, the ratio of kreatin nitrogen to total nitrogen was high.

On the last day which is recorded here, and three days before the death of the animal, 5.0 gm. of glutaric acid in the form of the sodium salt were administered subcutaneously. This was done for other purposes than those mentioned in this paper.

While the nitrogen output fell considerably, the output of kreatin was much increased, and hence one obtains a ratio on this day which is much higher than at any other period of the phlorizin poisoning. In this instance, with the administration of a substance, part of which was undoubtedly converted into carbonates, a distinct increase is observed in the kreatin excretion. That the increase was due to a change in the reaction of the urine is negatived by the fact that the urine was acid to litmus on this day

As Cathcart and Taylor have stated, there is apparently no connection between the acidosis observed in phlorizinized dogs and the amount of kreatin excreted. Hence the disturbance of carbohydrate metabolism giving rise to the acetone compounds cannot be the same as that leading to the excessive amounts of kreatin in the urine. This is also shown in these experiments. As will be seen from the tables, on one day, the 16th, during which the largest amount of kreatin was excreted, the amount of acetone compounds was practically the same as on the previous days.

The second animal is interesting, for while in very much better condition at the beginning of the experiment, she succumbed to the poison after five days' treatment.

The general features of the case are the same as those of the previous one. The glucose nitrogen ratio for the seventh and eighth days of the experiment indicates a total diabetes. As the notes of the case explain, the values for the ninth day are uncertain, because, aside from the fact that the animal died on this day, the urine was mixed with feces and vomit. It is possible that the low values obtained for kreatin are due to this cause.

What is noteworthy in this experiment is the steady rise in the ratio of kreatin nitrogen to total nitrogen. The process leading to the excretion of kreatin seems to outstrip the breakdown of protein. The kreatin output is about three times as prominent as in the previous experiment. This may be due to two causes, which have been also suggested as playing a part in the metabolism of uric acid. The increase in the output in this case may be due to increased formation of kreatin or to an inhibition of the processes whereby the kreatin formed in the processes of metabolism is prevented from being converted into urea. While we have no data which will allow us to differentiate between the two, it seems that as the kreatin excretion has become so prominent, an inhibition of the conversion of this substance into urea is more likely. The prominence of the kreatin excretion in this animal combined with its early death leads one to believe that there is some connection between the two. This excessive kreatin excretion has been observed by one of us in the days preceding death in cases of pneumonia [9] and typhoid fever in man.

With this unusual excretion of kreatin there are no features of the kreatinin output which call for comment; nor is the excretion of the acetone compounds essentially different from that found in the previous experiment.

In attempting to throw light on these processes of metabolism, one of us, with those who have worked with him in this laboratory, has devoted considerable attention to the excretion of sulphur, in the hope that by comparing the amount of sulphur excreted with that of nitrogen, one might be able to detect differences in the type of protein katabolized. It was with this object in view that estimations of the total sulphur output were made.

An inspection of the tables will show that no conclusion can be drawn regarding the type of protein katabolized as a result of phlorizin poisoning. It does not seem to differ in respect of its sulphur content from that used up in starvation.

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[9] Wolf and Lambert: Archives of internal medicine, 1910, v, p. 406.

It would therefore seem that the excretion of kreatin is not the result of the katabolism of a special type of protein, but is conditioned by unusual processes which affect the animal in fasting, and to a greater extent in phlorizin diabetes.

SUMMARY.

The excretion of kreatinin, kreatin, ammonia, the acetone compounds, and total sulphur has been examined in fasting and in fasting combined with total diabetes induced by phlorizin.

The amount of kreatinin increases during the glycosuria, but not at all in proportion to the breaking down of protein.

The kreatin excretion increases to such an extent in phlorizin diabetes that the ratio of kreatin nitrogen to total nitrogen steadily rises during the poisoning.

The processes which give rise to the acetone compounds are apparently distinct from those through which kreatin is excreted.

There is no relationship between the total sulphur metabolism and the acetone compounds or the kreatin.

STUDIES UPON THE TEMPERATURE COEFFICIENT OF THE RATE OF HEART BEAT IN CERTAIN LIVING ANIMALS.

By CHARLES G. ROGERS.

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I. Introduction.

SEVERAL years ago it was suggested to me by Professor Loeb that a study of the rate of heart action as influenced by temperature changes might prove to be of great value in determining the character of the underlying cause of cardiac contraction. It was impracticable at that time for me to undertake the work, and only within the past year has it been possible to pay any particular attention to it. In the mean time the problem has been worked upon to some extent by others. Up to the present time the results attained have not all been accounted for, and the problem in its deepest significance is still unsolved, though the answer may have been partly given.

Studies already made upon a number of physiological processes show that as the temperature of the active tissue is modified there is an associated change in the rate of the physiological process, which is, in some cases at least, to be accounted for upon the ground that the process is a purely chemical one, and that it therefore follows the same laws as do the chemical actions carried on in the laboratory. A study of many chemical reactions has revealed the fact that for an increase of 10° C. in temperature the rate of reaction is increased from 2 to 3.5 fold. For purely physical phenomena the increase is smaller, rarely reaching two and in some cases being actually less than one.

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[1] It is a pleasure in this connection to acknowledge the many courtesies received from the directors and staff of the Marine Biological Laboratory during the course of the work.

We are indebted to Snyder[2] and to Arrhenius[3] for two formulæ which make it possible for us to compare the constants of physiological processes with those of chemical reactions. The formula given by Snyder takes the form

$$Q_{10} = \left(\frac{K_1}{K_0}\right)^{\frac{10}{T_1 - T_0}}$$

in which Q_{10} is the coefficient of the increase in reaction velocity for a rise of 10° Celsius. The symbols K_1 and K_0 represent the rates of beat of the hearts studied at the temperatures T_1 and T_0 respectively, given in degrees Celsius.

Arrhenius has given us an empirical formula which takes the form

$$\text{Nat. Log}\,\frac{K_1}{K_0} = \frac{\mu}{2}\left(\frac{T_1 - T_0}{T_1 T_0}\right),$$

in which K_1 and K_0 represent the rates of heart action (or of chemical reaction) at the temperatures T_1 and T_0 respectively, in this case the temperatures being stated in degrees of the absolute scale. The symbol μ indicates a constant, the value of which for an ordinary chemical reaction is not far from 13,500, though it may vary according to the character of the reaction. For ordinary chemical reactions the value of Q_{10} usually lies between 2 and 3.5.

Many of the experiments made thus far have concerned the beatings of isolated hearts, working under decidedly artificial conditions in artificial solutions. For the sake of comparison, at least, it was deemed wise in this investigation to make observations upon hearts or pulsating vessels beating in the undissected animals.

There can be no doubt that the rate of the heart beat in the living, normal animal is the resultant of a large number of factors, which if kept at a constant relative value will result in definite rate of cardiac contraction. If any one of the factors be disturbed, there will be of necessity a disturbance of the rate of contraction and a new rate of contraction will be found which will meet the demands of the new conditions. Of these factors, it is evident that temperature is one.

We cannot presume to say that in dealing with an undissected animal and subjecting it to changes of temperature, we are of necessity modifying only the one factor, temperature. Nor has it yet been established that the temperature of the heart muscle or the pulsating vessel actually attains the same temperature as that of the medium in which the animal is immersed.

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[2] SNYDER, CHARLES D.: This journal, 1908, xxii, pp. 309–334.

[3] ARRHENIUS: Immunochemistry, Macmillan Co., 1907.

II. LITERATURE.

It hardly seems necessary to do more than briefly mention a few of the papers which have a bearing upon the general subject. Snyder[4] found that the rate of the heart beat of the Pacific terrapin is influenced by temperature changes in much the same way as chemical reactions; that the minimum of contraction occurs at 0° and the maximum rate between 35° and 37.5°. It was also noted in this paper that the coefficients vary considerably throughout the ranges of temperature studied, having a value as high as 10.2 for the temperatures 0° to 10° C., and gradually declining to 2.2 for the range between 10° and 20° C., and to 1.9 for the temperatures 20° to 30° C., and to 1 for the range 30° to 40° C. In a later paper Snyder[5] found that the heart beats of a mollusc, Phyllirrhoe, and of a crustacean, Maia verrucosa, behave in a similar manner. Robertson[6] found that the heart of Daphnia responded in a similar manner to changes of temperature. The papers of Martin, Martin and Applegarth, Langendorff, Langendorff and Lehmann as quoted by Snyder[7] indicate similar effects of temperature changes upon the hearts of dogs, cats, and rabbits. A third paper by Snyder gives additional data of a confirmatory character.

Riddle,[8] in reporting upon his work upon digestion in cold-blooded animals, shows that the process of digestion in these animals follows the same general trend. He assigns the increase in size of the coefficient of reaction velocity as zero is approached to a destructive effect exerted by the lower temperature upon the digestive secretions, and the decrease in the size of the coefficient at the higher temperatures to be due to an inhibitive or destructive effect of the temperature upon the ferment.

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[4] SNYDER, CHARLES D.: University of California Publications, 1905, ii, No. 15, pp. 125–146.

[5] SNYDER, CHARLES D.: This journal, 1906, xvii, pp. 350–361.

[6] ROBERTSON, T. B.: Biological bulletin, 1906, x, p. 242.

[7] SNYDER, CHARLES D.: This journal, 1908, xxii, pp. 309–334.

[8] RIDDLE, OSCAR: This journal, 1909, xxiv, pp. 447–458.

III. MATERIAL.

Up to the present time many of the animals experimented upon in work of this character have been chosen from among those rather high up in the animal kingdom, and are therefore among the more highly specialized forms. It is possible that on this account they may be less suited for such a study than some of the lower animals in which organic specialization, and doubtless cellular specialization as well, has not gone to so great an extent.

Again, the animals so far studied have been in the main those known as the warm-blooded—homothermous—animals, in which the normal physiological processes occur and continue only within a very narrow range of temperatures. Another great group of animals, the cold-blooded, as they are called — poikilothermous — includes those in which there is no normal stationary temperature for normal body processes, but in which there is a wide range of temperatures through which the physiological processes may occur. This group furnishes us with as good or better opportunities for determining the effects of changes of temperature upon physiological activities, for here the temperatures to which the animal is likely to be subjected during the course of experiment are the same as those which the animal might meet during the natural course of its existence, and to which it has become more or less adjusted through the experiences of preceding generations.

The first form chosen for this study was a small worm, Tubifex (sp.?). A large culture of these worms was obtained in the fall of 1909 from a small pond near the laboratory. They had been kept in small aquaria in the laboratory during the winter and had thrived well. At the present writing there are still hundreds of them available for work. For comparison with this form, during the work of the summer, another worm, Nereis virens, was chosen. Nereis does not thrive so well under the artificial conditions of the laboratory, so it was found desirable to obtain fresh supplies for each day's work.

The two other forms studied were chosen, not so much on account of their adaptability to the work at hand as because they were available when material was wanted and the things particularly desired were not. They proved, however, to be excellent material for the study of this problem. The material consisted of embryo toad-fish in which the circulation was well established and in which the yolk sac still formed a very conspicuous structure, and the embryos of the minnow Fundulus, studied as soon as the hearts had begun to beat and continuing until the beating of the hearts could be seen only with great difficulty on account of the rapid development of other organs and tissues.

IV. METHODS.

When the work was first undertaken, a number of experiments were made to establish methods of work which would allow sufficiently careful observations to be made and at the same time eliminate the greater sources of error. In so far as possible, the experiments of a given series were performed under conditions which were uniform except in the matter of temperature.

In the work upon Tubifex the particular worm to be studied was placed in a small beaker containing about 20 c.c. of water, and the beaker then floated in a bath which contained water of the desired temperature. In this way the water in the beaker soon came to have the same temperature as that in the bath, and the temperature of the worm presumably came to be closely approximated to that of the water in which it was placed. This water bath was connected by means of short rubber tubing with a warm stage upon the stage of the microscope, and the water of the bath allowed freely to run through the chamber of the warm stage. This stage was provided with a thermometer which was found to be sufficiently accurate for the work at hand. The temperature of the stage as indicated by its thermometer and the temperature of the water in the bath were kept closely approximated during the course of the experiment. After the specimen to be examined had been exposed to the temperature of the bath for a few minutes it was quickly transferred by means of a pipette from the beaker to a slide which had previously been placed upon the warm stage and which had the same temperature as the bath and the stage. By means of a stop watch the time required for twenty-five (usually) beats of the dorsal blood vessel at a given point was taken. From the data thus secured the rate of beat per minute was calculated. The method given above may be criticised on account of the danger that the temperature of the animals may become modified during the period given to observation, and that the temperature indicated by the warm stage thermometer does not accurately represent the temperature of the animals. These possibilities have been kept in mind, and while a re-examination of this part of the work may slightly modify the figures given in the tables, I think it very doubtful if there will be any important change. The method was used for the lack of a better one at the time.

The methods employed at Woods Hole in the study of Nereis, Fundulus, and the toad fish vary in some details from those described above. The specimens were placed, each in a finger bowl, separately numbered, in about 100 c.c. of sea water. The bowls were then placed for a period of about two hours in a bath of running sea water before making the first series of readings. The temperature of the running sea water was found to be very uniform for any two-hour period, though there were daily fluctuations. Each specimen of Nereis remained in the same finger bowl from the beginning to the end of the experiment. The eggs of Fundulus and of the toad fish were transferred at the time of observation to a small stender dish which could be placed upon the stage of the microscope. Care was always taken to avoid any change of temperature on the part of the eggs, in making the transfer. The heart beats to the number of twenty-five, fifty, or one hundred were then timed by means of a stop watch, and the eggs immediately replaced in the finger bowls from which they had been taken, there to remain until the next time for observation. To control the temperatures two thermostats, in which the finger bowls could be placed, were employed. The thermostat for the higher temperatures was warmed by means of gas flames, and the water kept constantly stirred by a series of fans operated by a hot-air engine. The thermostat for the lower temperatures was cooled by the addition of ice and the water stirred in the same manner as the first.

A number of attempts to immobilize the animals by means of curare and magnesium sulphate were made, but the results were unsatisfactory. The length of time through which the animals had to be under observation was too great for the continued action of either of the substances mentioned without very serious effects.

V. Possible Sources of Error.

In carrying out these experiments care has been taken to reduce the errors of experimentation to a minimum. All the temperature readings, except in the case of Tubifex, have been made with a standardized chemical thermometer reading to 1/10° C. In the case of Tubifex the stage thermometer employed has been checked by the side of the standard thermometer and has been found to have only small errors which would not serve to modify the general character of the result.

It was found during the course of the experiments that the specimens of Nereis were slow to adjust themselves to the changed temperature of the environment. This fact had not been apparent in the case of Tubifex, but for the sake of accuracy in the further work upon Fundulus and the toad fish full allowance was made for such a "latent period" as would permit of a complete adjustment to the new conditions. Even though the animals are recognized as adapted to life at wide ranges of temperatures, we must recognize that a certain definite period of time is required for the various physiological processes to become readjusted when any profound disturbance occurs in any one set of governing conditions. Snyder found that for the isolated hearts studied by him only a few minutes were required to establish a new rate of contraction after they had been subjected to the change of temperature. Robertson, also, in his study of heart rate in Daphnia, used a given temperature for only a short period of time. So far as the form used is concerned this may have been correct procedure. In the work here reported it was found that a very considerable period of time was required to assure complete adjustment to a new temperature. In the case of Nereis from one and a half to two hours was required before a complete adjustment could be depended upon.

Variations in the temperature of the surrounding medium may occur from time to time and of course would have an effect upon the accuracy of the observations. By the use of automatic regulators it has been found possible to maintain the temperature of the water of a given bath at a practically constant point during the period of experimentation. Even this does not meet the full requirement of the case unless the water in the vessel containing the animal under observation be also frequently stirred so that all parts of the liquid are of the same temperature. In the experiments reported in this paper it was found necessary frequently to stir the solutions in the manner suggested, as the temperature in different parts of the same beaker or finger bowl might vary as much as 1° Celsius if left undisturbed.

There are also physiological factors which tend to vary the rate of pulsation at any given temperature. Variation in the amount of oxygen supplied to the animals may occur from time to time. Accumulations of the products of excretion and of respiration take place and may affect the animal in respect to the rate of heart contraction. The importance of the nervous control of heart action in such forms as have been studied in this work is a matter we know little about at the present. In the two fish embryos studied it is a matter of doubt whether nervous connections with the heart have yet been established. In the other forms such connections may exist, but we do not know how effective they may be. It is the purpose of the author of this paper to continue the work along this line and determine if possible how the development of the nervous control may affect the rate of heart beat as influenced by changes of temperature.

VI. DATA FROM EXPERIMENTS.

In order that the general method employed in making the experiments may be apparent, I will quote from my original notes the data of a couple of experiments selected at random.

No. exp.	Temperature.	Seconds.	Beats counted.	Rate per minute.
45	21.5	85.8	25	17.48
	8.0	130.0	10	4.61
	21.9	78.0	25	19.23
	35.0	29.8	25	50.17
61	19.9	98.0	25	15.30
	30.0	45.0	25	33.33
	20.1	88.0	25	17.04
	7.0	127.0	10	4.72

Both of the above are taken from the records of the work upon Nereis. It does not seem necessary or wise to burden the paper with all of the data obtained, but rather to put the results into such form as will make them most useful.

The accompanying tables represent the facts as found in the study of this problem. In each case the number indicates the serial number of the animal experimented upon. When the same number occurs more than once in the tables of the same series, it will be apparent that the same animal was used for tests at both high and low temperatures. For the sake of convenience T_1 and T_0 as given in any table represent the higher and lower temperatures in degrees Celsius at which the rates K_1 and K_0 were determined. From the data thus obtained the values of Q_{10} and μ were calculated according to the formulæ of Snyder and Arrhenius and their values placed opposite.

TABLE I.

Lower limit.	Upper limit.	Q_{10}.	Average.	μ.	Average.
6	17	6.46		30190	
6	15	5.682	6.071	27920	29055
9	15.5	4.135		23094	
9.5	16	3.454		20238	
9	16	2.064	3.213	12810	18714
11	26	1.947		11328	
11.8	26	1.768		10086	
11.8	26	1.753		10147	10520
13	26	1.765		9562	
13.9	20	1.715		9287	
12.2	22	1.585		7751	
12.8	23.4	1.572	1.657	7664	8566
15.5	25	1.841		10500	
16	25	1.816		10270	
17	26	1.638		8469	
15	22.5	1.464		6489	
16	23	1.321		4770	
15	33.9	.9526	1.505	3971	8039

If we arrange the principal data collected from the experiments upon Tubifex according to the values of Q_{10} and μ as determined by our calculations, we find that the experiments tend to arrange themselves into a regularly descending series for the values of these two constants as we pass from 0° to the higher temperatures. Table I gives such an arrangement.

TABLE II.

No.	T_1	T_0	K_1	K_0	Q_{10}	μ
41	21.75	7.6	14.02	3.19	2.847	17301
42	21.65	8.0	16.69	4.56	2.587	15740
45	21.70	8.0	18.35	4.61	2.741	16699
47	21.75	7.8	17.22	4.91	2.457	14891
48	22.40	8.0	17.41	4.51	2.849	16876
49	22.40	7.9	18.48	4.05	2.849	17374
50	22.50	7.8	15.32	4.27	2.385	14422
51	22.50	7.8	18.23	4.54	2.574	15694
52	23.6	7.7	17.17	4.27	2.399	14573
53	22.5	7.6	18.25	4.28	2.674	16140
61	20.0	7.0	16.17	4.72	2.578	15542
62	20.1	7.0	10.03	4.03	2.141	12501
86	20.9	4.3	13.48	3.53	2.310	13680
87	20.9	4.9	11.30	3.75	1.003	11261
Average					2.525	15192

Table II shows the data collected from the experiments made upon Nereis at the lower ranges of temperature. The average values, $Q_{10} = 2.525$ and $\mu = 15{,}192$, indicate very clearly the character of the reaction. In a similar way the values of the constants for the upper ranges, calculated from data obtained in a series of more than forty sets of observations, give the averages $Q_{10} = 2.416$ and $\mu = 15{,}811$, values corresponding very fairly with those already given. It will appear later that in other forms there is not this striking similarity in the values of the constants for the upper and lower ranges.

In a similar way Tables III and IV indicate the results of the work

TABLE III.

No.	T_1.	T_0.	K_1.	K_0.	Q_{10}.	μ.
1	22.4	7.5	90.91	23.81	2.495	14898
2	22.4	7.5	103.4	25.00	2.593	15790
3	22.4	7.5	86.7	23.08	2.431	14715
4	22.4	7.5	100.7	22.22	2.757	16806
5	22.4	7.5	61.98	27.27	1.735	9131
6	22.4	7.5	101.4	25.00	2.559	15573
7	22.4	7.5	105.6	27.27	2.481	15058
8	22.4	7.5	107.2	23.08	2.803	17081
9	22.4	7.5	107.9	18.75	3.236	19464
16	22.3	5.3	131.6	18.51	3.170	18964
17	22.4	5.5	128.4	10.34	4.440	24534
18	22.4	5.6	117.6	21.58	2.744	16837
21	21.2	8.8	116.3	27.77	3.174	19150
22	21.2	8.7	98.68	29.76	2.609	15895
23	21.2	8.5	112.7	29.42	2.879	17516
24	21.4	8.5	101.4	25.25	2.938	17863
25	21.3	8.5	100.0	28.30	2.681	16340
26	21.8	4.0	126.1	14.85	3.325	19626
27	21.8	3.2	142.9	8.43	4.580	24777
28	21.8	3.4	136.3	11.76	3.787	21680
29	21.8	3.3	96.77	7.01	4.133	23115
30	21.8	3.3	130.4	9.03	4.234	23512
Average	21.5	6.55			3.081	18105

upon the heart of Fundulus. Here the averages of the constants as given at the ends of the tables indicate clearly that the acceleration of the heart beat decreases steadily as we pass from 0° upward toward 33°. This result is in harmony with the observations of others. Snyder found that in the case of the terrapin the value of Q_{10} for the temperatures $0° - 10°$ to be 10.2, from $10° - 20°$ to be 2.2, and from $20° - 30°$ to be 1.9.

TABLE IV.

No.	T_1.	T_0.	K_1.	K_0.	Q_{10}.	μ.
1	33.7	22.6	201.4	119.2	1.638	8946
2	33.7	22.6	206.9	135.8	1.460	6861
3	33.7	22.6	200.0	122.9	1.551	7951
4	33.6	22.6	202.7	124.1	1.562	8081
5	33.6	22.6	204.1	119.8	1.632	11051
6	33.6	22.6	207.7	123.8	1.600	8530
7	33.6	22.6	202.7	125.6	1.548	7887
8	33.6	22.6	197.3	131.6	1.445	6673
9	33.4	22.6	200.0	128.3	1.456	7470
11	34.1	22.5	209.8	129.8	1.513	7513
12	34.1	22.5	159.6	106.8	1.414	6287
13	34.1	22.4	205.4	120.2	1.581	8311
14	34.1	22.3	199.7	126.1	1.476	7066
15	34.1	22.3	206.9	137.6	1.413	6269
Average	33.78	22.53			1.521	7778

From a series of experiments made upon the hearts of embryo toad fishes we obtain the average values $Q_{10} = 2.221$ and $\mu = 13{,}216$ for the temperatures 9.9° to 20.70 and $Q_{10} = 1.883$ and $\mu = 11{,}121$ for the range of temperatures 20.7° to 30.6° C.

VII. Summary and Conclusions.

An examination of the data presented in the foregoing tables shows:

1. That the rate of contraction of the dorsal blood vessels of the worms and the hearts of the fishes studied is modified by the changes of temperature to which the animals are subjected.

2. The slower rates of contraction are always at the lower temperatures and the faster rates at the higher temperatures.

3. The values of Q_{10} and μ as constants of reaction velocity are higher for the range of temperatures near zero and gradually decrease as the temperature is increased.

4. The values of Q_{10} and μ for the different animals are not the same, though they are of the same general order of magnitude.

5. The average values of Q_{10} for each of the forms studied are such as would be expected if the heart beat were the result of a chemical reaction.

6. The variations in the values of the constants would seem to indicate that the reaction is not a simple one, and possibly that we are dealing with a series of reactions which at the different temperatures are affected differently. This part of the problem involves a further study.[9]

7. The increase in the value of the coefficient as zero is approached is entirely in harmony with the observations of Riddle upon the action of certain digestive enzymes, and lends force to a suggestion previously made by Loeb that the heart beat may be in fact the result of an enzyme action.

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[9] Since writing this sentence my attention has been called to the statement of Snyder: This journal, 1908, xxii, pp. 195–200, in which a similar suggestion is made. The conclusion offered here, arrived at entirely independently, serves to make the fact all the more certain.

ON THE ABSORPTION OF ALUMINIUM FROM ALUMINIZED FOOD.

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I. Introduction.

IT is a striking fact that although the employment of alum in food products is prohibited in all European countries, thousands of pounds of alum baking powder are annually sold and used in the United States. For many years biologists have been discussing the objections to the use of alum in food, especially to its use as a preservative and in the form of alum baking powder. While many contend that the ingestion of alum undermines the general health, some claim that this substance may be used with safety in the amounts ordinarily consumed. In view of the fact that legislative bodies, and public officials dealing with food products, are frequently confronted by this problem and because a number of prominent investigators are still endeavoring to solve it, the question may well be regarded as a " live " one.

About two years ago Professor J. W. Mallet of the University of Virginia conducted experiments on the solubility, in the stomach, of aluminium compounds contained in bread made with alum baking powder. On finding that a large proportion of such aluminium is soluble in the contents of a normal man's stomach, it seemed to Professor Mallet that, in accordance with our knowledge of the qualities of similar soluble salts, more or less of such dissolved aluminium must pass into the blood. If this were the case, he considered that the use of alum in food would be harmful.

Appreciating the desirability of special research in this connection, Professor Mallet suggested [1] that Professor Gies conduct experiments which would show definitely whether aluminium in aluminized food passes from the alimentary tract into the blood. Accordingly this investigation was undertaken at Professor Gies's suggestion. The entire series of experiments was carried out about a year ago under his personal supervision.

II. General Description of the Experiments.

Nature of the experiments. — The investigation consisted of fifteen experiments on dogs. Eleven of the experiments pertained to the passage of aluminium into the blood from aluminized food, and four experiments related directly to the passage of aluminium from the blood into the feces. In Experiments 1–9 alum was administered in powdered form in "meat-hash pills." In Experiments 10 and 11 biscuits baked with alum baking powder were fed. In Experiments 12 to 15, inclusive, aluminium chlorid, in physiological saline solution, was injected intravenously.

Animals and environment. — All the experiments were performed on full-grown, normal, healthy dogs. The animals, while under observation, were confined in cages of the kind now in constant use in this laboratory.[2]

Food. — The daily food of the animals with two exceptions (Experiments 10 and 11) consisted of a mixture of hashed lean beef, lard, cracker meal, bone ash, and water. The raw beef was taken from large supplies preserved in a frozen condition.[3]

In Experiments 10 and 11 biscuits prepared with alum baking powder were substituted for the cracker meal and for a portion of the beef hash. The biscuits, which were made by the process that

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[1] Private communication.

[2] Gies: This journal, 1905, xv, p. 403.

[3] Gies: *Ibid.*, 1901, v, p. 235; Proceedings of the Society for Experimental Biology and Medicine, 1908, vi, p. 27.

Professor Mallet employed in his experiments,[4] were baked with special thoroughness. The ingredients were mixed in the following proportions:[5]

Flour	550 gm.
NaCl	8 gm.
Sucrose	5 gm.
"Bob White" baking powder	16 gm.
Water	Enough to make a biscuit dough.

Methods for the quantitative determination of aluminium. — Before starting the experiments on the absorption of aluminium from the alimentary tract, I made a careful comparative study of the best methods for the determination of aluminium. After several trials of all the standard methods had been completed, the method adopted by the Association of Official Agricultural Chemists,[6] with a slight modification, was chosen. This method may be briefly described as follows:

Obtain an aliquot portion of the available acid solution (extract) and remove any contained silica. Mix the liquid with sodium phosphate solution in excess of what is required to form normal aluminium phosphate. Add ammonium hydroxid solution until a precipitate remains after thorough stirring. Then add hydrochloric acid solution, drop by drop, until the precipitate completely dissolves in the mixture. Heat the liquid to about 50° C., and mix with it at that temperature a considerable excess of 50 per cent ammonium acetate solution, and 4 c.c. of 80 per cent acetic acid solution. As soon as the precipitate of aluminium phosphate (mixed with iron phosphate) has sedimented, collect it on a filter, wash it with hot water, ignite it and then weigh the residue. Fuse the ignited material (mixed phosphates) with ten parts of sodium carbonate, dissolve the fused mass in dilute sulfuric acid solution, effect reduction with hydrogen sulfid, and finally determine the quantity of iron, if any is present, by the well-known permanganate method. In the same solution determine the phosphoric acid. To obtain the weight of Al_2O_3 subtract the sum of the combined weights of Fe_2O_3 and P_2O_5 from the weight of the mixed phosphates.

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[4] Private communication.

[5] The flour contained the merest trace of aluminium. The "Bob White" brand of baking powder is a "straight alum" powder. Our sample was purchased in the open market.

[6] U. S. Department of Agriculture, Bureau of Chemistry, Bul. 107, 1908, p. 177.

This method was followed precisely with one exception: instead of fusing the combined phosphates (after ignition) with sodium carbonate, the iron was determined by the Zimmerman-Rheinhardt method[7] in an aliquot portion of the original acid solution. The calculated amount of $FePO_4$ was subtracted from the combined weights of $AlPO_4$ and $FePO_4$. *Many determinations were made by this method on mixtures of pure solutions of ferric chlorid and alum, and perfectly accurate results were obtained.*

The oxidation of blood (Experiments 1–11) was effected by running the blood from a saphenous artery through a rubber tube directly into a tared Kjeldahl flask and weighing to determine the amount of available blood, then adding concentrated nitric acid solution and heating, slowly at first and finally boiling, until the solution was clear. A moderate excess of nitric acid solution was then added and the liquid boiled down to a small volume. The fluid was then treated with a fairly large volume of concentrated sulfuric acid solution for the expulsion of the nitric acid and to complete the oxidation of the residual organic matter. When necessary, small quantities of ammonium nitrate were added from time to time to facilitate the oxidation process.

The sulfuric acid mixture was boiled for at least two hours after it became colorless, in order completely to eliminate NO_2. The residue was then dissolved in water and made up to volume, and the iron and aluminium determined as described above. *The blood of dogs is normally free from aluminium, as this method showed.*

The feces (Experiments 12–15) prior to the precipitation of any aluminium contained in them, were incinerated in a platinum dish. The ash was dissolved in dilute hydrochloric acid solution. This solution was directly subjected to the analytic procedure described on page 96.

Anesthetic employed. — In order to avoid any disturbing effects of general anesthesia, a *local* anesthetic was employed in all the surgical operations. Less than 1 c.c. of a 2 per cent solution of cocain was used at any one operation. In no case did the dog exhibit any sign of pain.

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[7] Mixer and Dubois's modification of the Zimmerman-Rheinhardt method, Journal of the American Chemical Society, 1895, xvii, p. 405.

III. EXPERIMENTS SHOWING THAT ALUMINIUM PASSES FROM THE ALIMENTARY TRACT INTO THE BLOOD.

Eleven experiments were performed to determine whether aluminium passes from aluminized food into the blood.

A. **When aluminium was added to the food as powdered alum in meat-hash pills.**

Experiment 1. — The weight of the dog was about 24.5 kilos. He was given 0.9047 gm. of alum in order to administer an equivalent of 100 mg. of Al_2O_3.[8] Three hours after feeding, 800 gm. of his blood were taken. This quantity of blood contained the aluminium in 1.3 mg. of Al_2O_3. (The dog was used in Experiments 2 and 3.)

Experiment 2. — Eight days later, when the dog used in Experiment 1 had practically regained his normal weight, he was given 1.8094 gm. of alum (2 × 0.9047 gm.).[8] Five hours after feeding, 726 gm. of his blood were removed. This quantity of blood contained the aluminium in 1.9 mg. of Al_2O_3. (The dog was used in Experiment 3.)

Experiment 3. — Although the dog used in Experiments 1 and 2 had regained his normal weight within five days, no alum was given until eleven days after the termination of the previous experiment. At this time the dog received 3.6188 gm. of alum (4 × 0.9047 gm.)[8] Seven hours after feeding, 857 gm. of his blood were obtained. This quantity of blood contained the aluminium in 5.9 mg. of Al_2O_3.

Experiment 4. — A dog weighing 13.4 kilos was given 1.8094 gm. of alum (2 × 0.9047 gm.)[8] Six and one half hours later, 540 gm. of his blood were withdrawn. This quantity of blood contained the aluminium in 2.6 mg. of Al_2O_3.

Experiment 5. — A dog weighing 19 kilos was given 3.6188 gm. of alum (4 × 0.9047 gm.).[8] Seven hours later, 787 gm. of his blood were subjected to analysis. This quantity of blood contained the aluminium in 10.9 mg. of Al_2O_3.

Experiment 6. — A dog weighing 17 kilos was given 2.7141 gm. of alum (3 × 0.9047 gm.).[8] Five and three-quarters hours later, 736 gm. of his blood were analyzed. This quantity of blood contained the aluminium in 6.7 mg. of Al_2O_3.

Experiment 7. — A dog weighing 17.6 kilos was given 3.6188 gm. of alum (4 × 0.9047 gm.).[8] Six and one-half hours later, 738 gm. of his blood were collected. This quantity of blood contained the aluminium in 3 mg. of Al_2O_3.

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[8] This quantity of Al_2O_3 was selected empirically. It is less than the equivalent amount of aluminium which is often ingested by one person in the aluminized bread of an ordinary meal.

Experiment 8. — A dog weighing 13.4 kilos was given 0.9047 gm. of alum *daily for eight days.* On the eighth day, six hours after the last dosage, the animal was bled to death. One thousand and fifty-eight (1058) gm. of his blood were available. This quantity of blood contained the aluminium in 3 mg. of Al_2O_3.

Experiment 9. — A dog weighing 10 kilos was given 0.9047 gm. of alum *daily for eight days.* On the eighth day, five hours after the last dosage, the animal was bled to death and 996 gm. of his blood were secured, This quantity of blood contained the aluminium in 4.3 mg. of Al_2O_3..

B. **When aluminium was administered in biscuits baked with alum baking powder.** — In two experiments (10 and 11) the dogs ingested portions of the same supplies of meat, lard, and bone ash that were available for the previous experiments, but, instead of cracker meal, they were given alum baking powder biscuits, which were made of the ingredients specified in the summary on page 96. The biscuits were very thoroughly baked.

Experiment 10. — A dog weighing 13 kilos was given daily, *for ten days,* about 5 gm. of alum baking powder (in baked biscuits). On the tenth day he was bled to death and 650 gm. of his blood were oxidized. This quantity of blood contained the aluminium in 2.6 mg. of Al_2O_3.

Experiment 11. — A dog weighing 11.1 kilos was given daily, *for nine days,* about 4 gm. of alum baking powder (in baked biscuits). On the ninth day he was bled to death and 572 gm. of his blood were analyzed. This quantity of blood contained the aluminium in 1.5 mg. of Al_2O_3.

The essential data of Experiments 1 to 11, inclusive, are summarized in Table I.

These results make it evident that aluminium passes into the blood in considerable amounts from alum in the diet. They also show that aluminium does not tend to accumulate in the blood.

These analytic data apply, of course, to the blood only at the moment of its removal from the body. Under the conditions of the experiments, it is certain that aluminium passed into and from the blood in much larger total quantities than those indicated. In Experiments 1 to 7, inclusive, the quantities of available blood ranged from 2.9 per cent to 4.3 per cent of the body weight. From three to four times as much aluminium as the quantity shown in the table was therefore contained in the whole volume of blood of the corresponding animal at the moment of bleeding (Experiments 1–7).

TABLE I.

Data showing the Passage of Aluminium from the Alimentary Tract into the Blood.

Exp. No.	Weight of dog.	Dose of alum.	Time between dosage and bleeding.	Weight of blood taken.	Total weight of $FePO_4 + AlPO_4$	Weight of $AlPO_4$.	Weight of Al_2O_3 from the total amount of blood taken.[2]
	kilos	gm.	hours	gm.	gm.	mg.	mg.
1	24.5	0.9047	3	800	0.9644	3.2	1.3
2	24.4	1.8094	5	726	0.8820	4.5	1.9
3	24.5	3.6188	7	857	0.9450	14.1	5.9
4	13.4	1.8094	6½	540	0.7650	6.0	2.6
5	19.0	3.6188	7	787	1.0170	26.0	10.9
6	17.0	2.7141	5¾	736	1.3980	15.0	6.7
7	17.6	3.6188	6½	738	0.8960	7.0	3.0
8	13.4	0.9047	6	1058	1.1330	7.1	3.0
9	10.0	0.9047	5	996	1.0285	10.5	4.3
10	13.0	5[1]	6	650	0.7854	6.2	2.6
11.	11.1	4[1]	7	572	0.5136	3.6	1.5

[1] Alum baking powder in biscuits. [2] See the paragraph on page 99.

The results of Experiments 10 and 11 show clearly that the aluminium in alum baking powder is not rendered wholly insoluble in the bread-baking process. An amount of aluminium equal to that recovered after the administration of plain alum in powdered form (Experiments 1 to 9 inclusive) was found in the blood of the dogs that received the alum baking powder biscuits.[9]

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[9] The reader is again reminded of the fact that special care was taken to subject the biscuits to very thorough baking.

IV. EXPERIMENTS SHOWING THAT ALUMINIUM PASSES FROM THE BLOOD INTO THE FECES.

The results of the foregoing experiments suggest that aluminium was not only absorbed from the gastro-intestinal tract and distributed throughout the body in the blood, but also eliminated fairly rapidly from the circulation. In considering the matter of its excretion from the body, we concluded to ascertain, first, whether aluminium passes into the feces after the parenteral introduction of aluminium chlorid.

TABLE II.

DATA SHOWING THE PASSAGE OF ALUMINIUM FROM THE BLOOD TO THE FECES.[1]

Exp. No.	Weight of dog.	Amount of aluminium injected.	Dry weight of feces eliminated during the 3 days following the injection.	Amount of aluminium found in the feces during the 6 days previous to the injection.	Amount of aluminium found in the feces during the 3 days following the injection.	Percentage of the injected aluminium that was recovered.
	kilos	mg. of Al_2O_3	gm.		mg. of Al_2O_3	per cent
12	10.0	9	67.5	None	1.0	11.11
13	9.4	18	64.0	None	1.1	6.11
14	8.2	36	47.5	None	1.9	5.27
15	9.2	82.8	56.0	None	4.6	5.55

[1] A dilute solution of Al_2Cl_6 was injected into a saphenous vein.

The animals selected for four experiments in this connection were normal, vigorous dogs weighing between 8 and 10 kilos. The daily diet was similar to that for the dogs in the first nine experiments. In each case the animal was kept on our standard diet for about ten days before the feces were collected for analysis. Thereafter the feces for the six days previous to the introduction of aluminium were carefully analyzed for aluminium. *None was found in any instance in the feces passed prior to the injection.*

The aluminium salt chosen for intravenous injection was aluminium chlorid, since it is probable that aluminium chlorid is produced from aluminized food in the stomach. A dilute solution, containing the equivalent of 0.9 of a milligram of Al_2O_3 per cubic centimetre, was very *slowly* injected into a saphenous vein.[10] That a portion of the injected aluminium passed from the blood into the feces under these conditions can be seen from the results of our four experiments as summarized in Table II.

V. GENERAL CONCLUSIONS.

The foregoing results justify the following conclusions:

1. When alum was administered in aluminium-free food to dogs, or when dogs ingested biscuits baked with alum-baking powder, aluminium in comparatively large amounts promptly passed into the blood.

2. Absorbed aluminium circulated freely, but as it did not show any pronounced tendency to accumulate in the blood, its full effects must have been registered outside of the circulation.

3. When aluminium chlorid was administered intravenously, from 5.55 per cent to 11.11 per cent of the aluminium passed from the blood into the feces during the three days immediately after the injection. Whether the aluminium passed directly through the walls of the intestine or was excreted by the liver, or whether both channels (or others) were followed, has not yet been ascertained.

It is Dr. Gies's purpose to extend this study.[11]

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[10] The wound healed promptly in each case.

[11] GIES: Proceedings of the American Society for Pharmacology and Experimental Therapeutics, Journal of pharmacology and experimental therapeutics, 1911, ii, p. 403.

THE EFFECT OF PARATHYROIDECTOMY UPON METABOLISM.

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I. Introduction.

THIS investigation was undertaken in the hope that a comparative study of urinary composition, before and after parathyroidectomy, would reveal any metabolic disturbances that result from parathyroid insufficiency. The excretion of the more important nitrogenous constituents of the urine, before and after parathyroidectomy, was first studied. No marked differences were observed. This negative result led to a more complete study of the excretory products under such experimental conditions, in the course of which a very marked change in the phosphorus metabolism was noted. Special attention was then given to the elimination of phosphorus after parathyroidectomy. The excretion of ammonia in the urine was also further investigated.

In spite of the great amount of research on the physiology of the parathyroids,[1] comparatively little has been done to determine the influence which their removal exerts upon metabolism. Most of the previous experiments in this connection have been performed upon completely thyroidectomized animals, and it is difficult to determine which of the observed effects were due to the removal of the parathyroid glands.

In 1895 Dutto and Lo Monaco[2] noted a diminished nitrogen excretion in the urine of a dog after thyroidectomy, but no importance can be attached to this observation because the nitrogen excretion had been decreasing steadily before the operation and, at that time, probably had not reached the level of nitrogen ingestion.

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[1] For a review of the literature, see Biedl: Innere Sekretion, Berlin, 1910.

[2] Dutto and Lo Monaco: Archives italiennes de biologie, 1895, xxiv, p. 196.

In the same year Roos[3] reported the results of a metabolism experiment upon a thyroidectomized dog. Tetany was observed on the second day after the operation, but the dog partially recovered after ingestion of sheep thyroid, and lived a little more than a month, with a very mild degree of tetany. The symptoms then became more pronounced and death soon followed. The urine was collected daily for twenty days after the thyroidectomy. During the two days immediately following the operation the amount of nitrogen in the urine was increased while the chlorids and phosphates were diminished. Averages for the periods show an increased excretion of nitrogen and chlorids, and a diminished excretion of phosphates, after thyroidectomy.

Ducceshi[4] reported an increased sulfur output and a higher ratio of neutral sulfur to total sulfur in the urine of dogs after thyroidectomy. Ducceshi's results are of doubtful value, however, because the analyses upon which he based these statements were made on only occasional specimens of urine, and most of the dogs ate very little after the operations.

Verstraeten and Vanderlinden[5] studied the nitrogen excretion after thyroidectomy in two dogs and a cat. The two dogs developed tetany; the cat did not, but died in a state of cachexia fifty-two days later. The amounts of nitrogen in the urines of the dogs after the operation were very much greater than the normal. The output of nitrogen in the urine of the cat diminished gradually but steadily until the death of the animal.

A number of experiments upon dogs, cats and rabbits were made by Ver Ecke,[6] who found that, after thyroidectomy, there was a decreased excretion of urea, which he estimated by the hypobromite method. Urinary phosphate was diminished and chlorid was generally increased in amount. The diminished phosphate excretion was noted in all the experiments, but was most marked in those in which tetany was also observed.[7] Ver Ecke's work has not received the attention it deserves. The fact that intoxication by a product of metabolism may be indicated by a diminished excretion of the same has apparently been overlooked by workers in this field.

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[3] Roos: Zeitschrift für physiologische Chemie, 1895, xxi, p. 19.

[4] Ducceshi: Archives italiennes de biologie, 1896, xxvi, p. 209; Lo sperimentale, 1896, l, p. 190.

[5] Verstraeten and Vanderlinden: Mémoires couronnés de l'académie royale de médecine de Belgique, 1894, xiii.

[6] Ver Ecke: Archives internationelles de pharmacodynamie, 1898, iv, p. 81.

More recently Coronedi and Luzzatto [8] noted an increase of the ammonia in the urine of a dog after removal of the thyroids. Underhill and Saiki [9] also found a high ammonia output in the urine of fasting dogs after complete thyroidectomy. The excretion of creatinin, creatin and allantoin was about the same as in normal fasting dogs. A few analyses showed a low phosphorus excretion, which Underhill and Saiki regarded as possibly indicating a diminished nuclear metabolism.

An increased ammonia excretion after thyroparathyroid- and parathyroidectomy has also been observed by MacCallum and Voegtlin,[10] Berkeley and Beebe,[11] and Frouin.[12] The last-named author also reported an increased excretion of carbamic acid, which he considered the causal agency in the production of tetany. In view of the fact that the existence of carbamates in any but strongly alkaline urines is open to question, it is very much to be regretted that Frouin did not give the figures he obtained nor describe the method he used for the estimation of carbamic acid.

MacLeod and Haskins [13] did not find carbamates in the urine of normal dogs. Ellinger [14] states that it is impossible to decide whether the carbamate ion, as such, is excreted by the kidneys, or is formed in the urine as a consequence of the simultaneous presence of ammonium salts and soluble carbonates.

MacCallum and Voegtlin [10] noted an increased excretion of calcium from dogs after removal of the parathyroids. Cooke[15] could not corroborate their findings, but reported an increased output of magnesium.

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[7] VER ECKE'S paper had been overlooked in a study of the literature that was made before the beginning of this investigation, and was not discovered until the literature was again reviewed after the experimental work had been concluded.

[8] CORONEDI and LUZZATTO: Archives italiennes de biologie, 1907, xlvii, p. 286.

[9] UNDERHILL and SAIKI: Journal of biological chemistry, 1908, v, p. 225.

[10] MACCALLUM and VOEGTLIN: Journal of experimental medicine, 1909, xi, p. 118.

[11] BERKELEY and BEEBE: Journal of medical research, 1909, xx, p. 149.

[12] FROUIN: Comptes rendus, 1909, cxlviii, p. 1622.

[13] MACLEOD and HASKINS: This journal, 1905, xii, p. 444.

[14] ELLINGER: OPPENHEIMER'S Handbuch der Biochemie des Menschen und der Tiere, Jena, 1910, iii, p. 569.

MacCallum and Voegtlin[10] noted a high content of ammonia in the blood of a dog in tetania parathyreopriva as compared with that of a normal dog. The same condition, as well as a diminished ammonia-destroying power of the liver, was found by Carlson and Jacobson[16] in cats and foxes in tetany after complete thyroidectomy. These results, together with the fact that the tetany of ammonia intoxication resembles that observed after parathyroidectomy, led Carlson and Jacobson to believe that the latter type of tetany may be due to a decreased destruction, by the liver, of ammonia and similar substances, which then accumulate in the blood. This view is strengthened by the results obtained by Miss Jacobson.[17] She found that the amount of ammonia in the blood of cats and dogs, in which the intravenous injection of ammonia had produced symptoms comparable to those observed after removal of the parathyroids, was about the same as that present after thyroparathyroidectomy.

II. Experimental.

Nature and conduct of the experiments. — We conducted six metabolism experiments on as many dogs weighing from 4.7 to 11.7 kilos. The animals were kept in some of the metabolism cages now in regular use in the laboratory of biological chemistry.[18] The food consisted of a mixture of hashed lean meat (prepared according to the method described by Gies[19]), cracker meal, pure leaf lard and water, which was given in one meal. Bone ash, which is regularly added to the diet of dogs in the biochemical laboratory, was excluded from this mixture. Although Steel and Gies[20] and Lothrop[21] have shown that there is ordinarily no appreciable absorption of calcium or of phosphoric acid from ingested bone ash, it was deemed best to avoid the complications that would have resulted from possible exceptions to the rule. Kaolin (Eimer and Amend's acid-washed) and infusorial earth (Eimer and Amend's finest powdered) were used instead of bone ash to prevent diarrhea. For this purpose infusorial earth was found to be much superior to kaolin and very nearly as effective as bone ash.

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[15] Cooke: Journal of experimental medicine, 1910, xii, p. 45.

[16] Carlson and Jacobson: This journal, 1910, xxv, p. 403.

[17] Jacobson: This journal, 1910, xxvi, p. 407.

[18] Gies: This journal, 1905, xiv, p. 403.

[19] Gies: *Ibid.*, 1901, v, p. 235; Proceedings of the Society of Experimental Biology and Medicine, 1908, v, p. 27.

[20] Steel and Gies: This journal, 1907, xx, p. 343.

[21] Lothrop: This journal, 1909, xxiv, p. 297.

The dogs were fed daily at 9 A. M., and the twenty-four-hour periods ended at 9.15 A. M. The cages were washed daily with distilled water, and the urine and cage washings were made up to a uniform volume. Thymol was used as a preservative, and the urines were kept in a refrigerator until the estimation of the nitrogenous constituents had been completed.

Analyses. *Time relationships.* — In the first three experiments (Tables I, II, and III) the ammonia was determined each day as soon as the twenty-four-hour urine was available. The estimations of total nitrogen, urea, and creatinin in the assembled urines were begun promptly after the appearance of symptoms of parathyroid insufficiency, and were completed as soon thereafter as possible. In a number of the assembled urines most of the creatinin had apparently been changed into creatin, since the figures obtained for the former were very low. Consequently, determinations were made of the sum of the creatin and creatinin as creatinin.[22] Phosphate and phosphorus were determined a few weeks later.

In the fourth experiment (Tables IV–V) the estimations of the acidity, ammonia, creatin, and creatinin were begun within an hour of the close of the corresponding period. The urea was determined on either the day of elimination or the next day. Purin substances were determined within five days of the date of urine collection. The sulfate, sulfur, chlorid, phosphates, phosphorus, and total nitrogen were determined soon after the close of the experiment.

In the fifth and sixth experiments the determinations of ammonia and inorganic phosphate were begun immediately after the close of the period. The total nitrogen and phosphorus of the urine, and the phosphorus of the feces, were determined shortly after the close of the experiment.

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[22] A change of creatinin into creatin in *acid* dog urines preserved with thymol has repeatedly been observed by the author and by other workers in this laboratory. As much as half of the creatinin may be transformed into creatin within a week.

Methods. — The analytical methods are indicated briefly in the appended summary:

Total nitrogen: Kjeldahl-Gunning method.

Urea: Benedict method,[23] which had been found by previous experiment upon dog urines to give results that were from 0.3 to 1.5 per cent lower than those obtained by the Folin method.

Ammonia: Steel's modification of the Folin method.[24]

Purins: Kruger-Schmidt method, with the modification suggested by Benedict and Saiki.[25]

Creatinin and creatin: Folin methods.

Total sulfur: Benedict method.[26]

Inorganic and total sulfates: Folin methods.[27]

Phosphate: In the first four experiments by titration with uranium acetate, with potassium ferrocyanide as the indicator; in the last two experiments by precipitation with magnesium citrate solution as described by Mathison.[28] The precipitate produced by magnesium citrate and ammonium hydroxide in the urine of normal dogs is crystalline and filters readily. In the urine of parathyroidectomized dogs, however, the precipitate is contaminated with a gelatinous substance which makes filtration difficult and thorough washing impossible. If, however, this precipitate is filtered from the bulk of the liquid and is then treated with hot, dilute hydrochloric acid solution, the ammonium magnesium phosphate is dissolved. From the solution thus obtained, the phosphate can be reprecipitated by means of magnesium citrate solution in the usual manner. The interfering substance does not dissolve in the dilute acid. It does not contain an appreciable amount of phosphorus.

Phosphorus: In the first four experiments by the Liebig alkali fusion process, precipitation with ammonium molybdate solution and reprecipitation as NH_4MgPO_4, filtration on a Gooch crucible and weighing as pyrophosphate; in the last two experiments, by oxidation with sulfuric and nitric acids according to Neuberg,[29] filtration from the silica (in the case of the feces), precipitation with ammonium molybdate solution, and further as above.

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[23] Benedict: Journal of biological chemistry, 1910, viii, p. 405.

[24] Steel: Journal of biological chemistry, 1910, viii, p. 365.

[25] Benedict and Saiki: Journal of biological chemistry, 1909, vii, p. 27.

[26] Benedict: Journal of biological chemistry, 1909, vi, p. 363.

[27] Folin: Journal of biological chemistry, 1906, i, p. 131.

[28] Mathison: Biochemical journal, 1909, iv, p. 233.

Acidity: Folin method.

Chlorid: Volhard-Arnold-Salkowski method.

Tenth-normal solutions of sulfuric acid and sodium hydroxide were used in the titrations. All determinations, except those of acidity, creatinin, creatin, and purin substances, were made in duplicate, unless otherwise noted.

The surgical operations were performed in the laboratory of pathology by Dr. W. G. MacCallum. The animals were completely anesthetized with ether. Strict asepsis was observed, and the wounds healed by primary union.

III. PROTOCOLS OF THE EXPERIMENTS.

First experiment. — Beginning on March 29, 1910, a young fox terrier, weighing 4.7 kilos, was daily fed a mixture of 85 gm. of hashed meat, 29 gm. of cracker meal, 16 gm. of lard, 7 gm. of kaolin, and 150 c.c. of distilled water. Four parathyroids were removed at 10.30 A. M., April 7, 1910. Because the operation was soon to be performed the dog was not fed at the usual hour (9 A. M.) but at 7.45 P. M., at which time recovery was apparently complete. Nothing unusual was noted on the following day. On the morning of April 9 the dog ate only a small portion of the food. Shortly afterwards a slight tremor was noticed. At 11 A. M., by means of a large spatula, the remainder of the food was placed in the dog's mouth and was swallowed. The tremor continued all day. On the following morning the dog would not take food from the dish and was fed from a spatula as on the previous day. A mild degree of tetany was present during the day. On the following morning there was no tetany, but, as the dog was in very poor condition, he was killed with chloroform.

Second experiment. — Beginning April 10, 1910, a young brown-and-white mongrel, weighing 5.7 kilos, was daily fed a mixture of 106 gm. of hashed meat, 28 gm. of cracker meal, 17 gm. of lard, 7 gm. of kaolin, and 160 c.c. of distilled water. Parathyroidectomy was performed at 10.30 A. M., April 19. The dog recovered rapidly and at 2 P. M. ate his food eagerly. The first indications of tetany appeared at 10.30 A. M.,

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[29] NEUBERG: Zeitschrift für physiologische Chemie, 1903, xxxvii, p. 115.

April 21, the tetany rapidly increasing in severity and reaching a maximum at about 11 A. M. that day. At 2.30 P. M. the dog's condition seemed slightly improved, but death occurred at about 2.45 P. M. Sixty c.c. of urine were obtained from the bladder. This represented the period from 9 A. M. to 2.45 P. M. — practically six hours.

Third experiment. — Beginning April 15, 1910, a young mongrel weighing 9.25 kilos, was daily fed a mixture of 139 gm. of hashed meat, 46 gm. of cracker meal, 28 gm. of lard, 10 gm. of kaolin, and 270 c.c. of distilled water. He was fed at the usual hour (9 A. M.) on April 22. At 3 P. M. four parathyroids were removed. Recovery was rapid. Food was taken eagerly on the 23d and 24th. At 1 P. M., April 24, a faint tremor of the head and shoulder muscles was noted. The tetany gradually grew more severe, general convulsions appearing at about 5.45 P. M. At 6.10 P. M. the dog was quiet, only a very slight tremor was noted, and respiration was 30 per minute; at 6.20 P. M. it was only 15. At 6.25 P. M. respiration ceased. The heart continued to beat two minutes longer. Two minutes after the heart had apparently ceased beating, the left leg, previously extended, was flexed against the abdomen and again extended. The entire movement took about thirty seconds. The tail continued to move for several minutes. An autopsy was conducted immediately. All the abdominal blood vessels and the spleen were very much congested. Otherwise nothing abnormal was observed. The urine in the bladder was expressed and added to that previously voided.

Fourth experiment. — Since two of the dogs in the first three experiments died very shortly after the onset of tetany, it was thought advisable to feed less meat in this experiment, as it is the general opinion among observers of parathyroid tetany that a meat diet tends to increase the severity of the symptoms and to hasten their appearance. Accordingly a male black-and-tan mongrel, weighing 11.13 kilos, was daily fed the following mixture, beginning May 27, 1910: 56 gm. of hashed meat, 90 gm. of cracker meal, 33 gm. of lard, 12 gm. of kaolin, and 385 c.c. of distilled water. Parathyroidectomy was performed at 11 A. M., June 5. The dog was fed that day at 4 P. M.

At 8.30 A. M. the next morning (June 6) the dog vomited about 30 c.c. of a mucous liquid containing a few small pieces of undigested meat. At 10 A. M. he was fed. No urine was voided until 8.30 A. M. on June 7. The dog was fed at 9 A. M. on that day. Two hours later a very faint tremor was noticed. This increased in severity very slowly, the animal being kept under close observation until 11 P. M. At 8.15 the following morning (June 8) the symptoms were more marked, but the food was taken at the usual hour (9 A. M.). His condition gradually grew worse. At 1 P. M. there was marked hyperpnea, which continued, with severe tetany, during the afternoon. He quieted somewhat at about 6.15 P. M., and twenty-five minutes later vomited about 300 c.c. of a slimy liquid containing several pieces of undigested meat. The twitching gradually grew less marked, at midnight being less severe than at 8 A. M. At midnight he drank 100 c.c. of water. The following morning (June 9) there was no tetany, but the dog was very quiet. He did not eat the last of his food until 5 P. M.

On June 10 the food and an additional 150 c.c. of water were taken at the usual hour. Slight twitching began at 11.30 A. M. and gradually grew more marked. The limbs were rigidly extended at 5 P. M. and remained so until after 11.45 P. M. At 2.30 A. M. (June 11) the dog was noticeably recovering from the attack. At 8.45 A. M. that day there was comparatively little twitching, but food was declined. The dog drank about 100 c.c. of water but vomited it, mixed with a few food particles and a little bile, about thirty minutes later. The twitching had disappeared at 6 P. M., but the dog would not eat. The food was then placed in his mouth with a large spatula and was swallowed. Half was given at 6 P. M. and half at 9 P. M. At the latter time the dog urinated. At 11 P. M. he drank about 400 c.c. water. At 10 A. M. the next morning (June 12) he was in violent tetany. During the night he had vomited about 300 c.c. of liquid, contaminating any urine that might have been voided after 9 P. M. The tetany grew less marked during the day, disappearing before 10 P. M. The day's food was then proffered, but the dog would not eat it voluntarily. He was then fed from a spatula as on the previous day. At 11.30 P. M. he was quiet and the food had been retained.

At 7.20 on the following morning (June 13) a mild tremor was noted. Seventy-five c.c. of bile-stained vomit were found in the cage, but no urine had been voided. The cage was cleaned and at 7.45 the dog urinated. Absence from the laboratory made it impossible to close the period at the usual hour. At 1.20 P. M. there was some additional urine in the bottle and the daily period was now closed. Shortly afterward the dog drank 200 c.c. of water. At 3 P. M. he rejected food and an attempt at " forced " feeding was unsuccessful. The tetany had been increasing in severity during the day. At 9 A. M., June 14, there was fairly general twitching of the muscles. The dog would not take food but drank 150 c.c. of water. At 11.30 A. M. he urinated and the daily period was closed. At 5 P. M. tetany was more severe. The next day (June 15) there was marked twitching of the muscles. He rejected food but drank water. On the morning of the 16th the dog was in slight tetany. The urine in the bottle underneath the cage was contaminated with fluid feces. At 10 A. M., 494 gm. of raw egg were given by stomach tube. A considerable quantity was vomited between 1.15 and 2.15 P. M. The dog was chloroformed at 3 P. M.

Fifth experiment. — A young female mongrel, weighing 9.1 kilos, which had been daily fed a mixture of 105 gm. of hashed meat, 30 gm. of cracker meal, 25 gm. of lard, 15 gm. of infusorial earth, and 250 c.c. of distilled water, for several days, was subjected to the usual operation on Dec. 5, 1910. Four typical parathyroids were removed. The dog recovered promptly and ate the day's ration greedily at 4.30 P. M. An additional quantity of water (150 c.c.) was given at this time, as the dog appeared to be very thirsty. The dog was fed at the usual hour (9 A. M.) on December 6 and 7. No symptoms of tetany were noticed until noon, on December 7, when the animal appeared to be ill at ease, brushing her forehead with her paws in the manner characteristic of parathyroidectomized dogs. At 3.15 P. M. a faint tremor was noticed. This increased in severity, and slowly reached a maximum at 8 P. M., then declined somewhat. Tetany was present all day on December 8. Food was declined, but when placed in the mouth was swallowed. In this manner about three fourths of the ration were given. The dog then rejected the rest of the food. The same condition of tetany was present throughout the following day (December 9). No food was taken on this day. Tetany was present on the morning of December 10, and the metabolism experiment was then concluded. The dog was chloroformed later in the day.

Sixth experiment. — A young, full grown, male fox terrier weighing 10.7 kilos was fed the following mixture daily, beginning Jan. 11, 1911: 164 gm. of hashed meat, 50 gm. of cracker meal, 32 gm. of lard, 18 gm. of infusorial earth, and 350 c.c. of water. Parathyroidectomy was performed at 10 A. M., January 19. Recovery was not very prompt, the dog suffering from a chill all afternoon. At 10.30 P. M. he was quite normal and ate the day's food ravenously. The next morning he was perhaps a little quieter than was usual, but otherwise was apparently normal. He ate his food eagerly. At 2 P. M. very faint twitching was noticed. This was quite marked at 3 P. M. At this time there was also some salivation. The dog was then anesthetized with ether and bled to death from a carotid artery. The bladder was emptied and the urine thus obtained was added to that previously voided.

IV. DISCUSSION OF RESULTS.

The analytic data are given in Tables I–IX.

Total nitrogen. — The excretion of nitrogen remained unchanged for a short time after the operation, but a day or two later it was, in every case, except in the fifth experiment, raised above that observed before parathyroidectomy. This increase was not uniform but ranged from about 10 per cent to over 100 per cent. It is difficult to believe that the food which these dogs ate provided sufficient energy for the very great amount of work performed in tetany. It is probable, therefore, that the high nitrogen excretion was due largely to the increased destruction of body protein which ensued to supply the deficiency.

Ammonia. — In order to make comparison more convenient, the figures representing the percentage of ammonia nitrogen in the total amount of urinary nitrogen have been brought together in Table VIII.

With only two dogs (second and third experiments) was an increased ammonia ratio observed before the appearance of tetany. The increases were rather slight. In the one case (third experiment), during the control period the ammonia nitrogen varied between 3.85 per cent and 4.70 per cent, averaging 4.42 per cent, of the total nitrogen. On the day of operation it fell to 3.39 per cent, but it rose to 6.02 per cent on the following day. In the second experiment the percentage of nitrogen as ammonia on the day of the operation dropped below the minimum of the control period and on the following day rose 1.04 per cent above the maximum. On the second day, however, there was a very marked fall in the ammonia ratio. As the dog had been fed that morning, this might have been due to the secretion of hydrochloric acid into the stomach.

In the first, fourth, and fifth experiments the ammonia ratio rose slightly above the normal with the appearance of tetany. In two of these experiments it fell, on the second day, to well within the normal limits. In the one case (first experiment) this may have been due to recovery. This animal displayed rather mild symptoms, which disappeared completely before the close of the second day after the operation. It is possible that an additional parathyroid had hypertrophied sufficiently to compensate for the removal of the others, or that the animal had in some other manner become immune to the effects of the parathyroidectomy, and that the poor condition of the dog, as was mentioned above, happened to be an accidental circumstance.

In the fourth experiment the very marked fall in the ammonia ratio on the third day after operation may have been due to the considerable loss of hydrochloric acid with the vomit on that day (June 8). The increased ammonia ratios for the later days of this experiment are of little significance. These increases were not very great. Besides, the dog was in severe tetany most of this time and received comparatively little food.

TABLE I.

Urinary Data. First Experiment.

1910 April	Weight.	Vol.	Sp. gr.	Reaction.	Nitrogen		
					Total	Urea.	NH_3.
	kilos.	c.c.		litmus.	gm.	gm.	gm.
4	4.70	140	1029	Amphot.	3.378	2.830	0.195
5	4.69	150	1026	Amphot.	3.248	2.780	0.177
6	4.70	136	1027	Amphot.	3.085	2.725	0.183
7	4.70	176	1025	Amphot.	3.707	3.208	0.156
8	4.64	65	1060	Amphot.	3.554	3.077	0.155
9	4.64	96	1037	Amphot.	3.541	2.949	0.160
10	4.64	110	1044	Amphot.	4.074	3.062	0.289
11	4.38	156	1031	Amphot.	3.457	3.000	0.201

The very slight rise in the ammonia ratio on the first and second days of tetany in the fifth experiment was followed by a more marked increase on the third day. However, the animal received no food on this day, and the urinary nitrogen was only about half of the usual amount. Very probably the increased proportion of nitrogen excreted as ammonia was due to this fasting condition.

In the last experiment, neither on the day of the operation nor during the six hours of the following day that intervened before the close of the experiment, was the ammonia ratio as high as the average for the control period, even if the rather high figure for the last day of this period be omitted from the calculation. Indeed, the ammonia ratio on the day of the operation was considerably lower than on any other day. The ammonia ratio for the day after the operation was lower than that for any control day but one.

TABLE. I.

URINARY DATA. FIRST EXPERIMENT.

Nitrogen.	Phosphorus in phosphate.	Percentage of total nitrogen.				
Creatinin and creatin.		Urea N.	Ammonia N.	Creatinin and creatin N.	Undetermined nitrogen.	Remarks.
gm.	gm.	per cent.	per cent.	per cent.	per cent.	
0.110	0.177	83.79	5.77	3.26	7.18	
0.106	0.183	85.59	5.46	3.28	5.67	
0.099	0.160	85.07	5.94	3.22	5.76	
0.114	0.175	86.54	4.22	3.86	5.38	
0.123	0.110	86.60	4.35	3.46	5.74	Operation at 10.30 A. M. April 7.
0.141	0.086	83.25	4.53	3.98	8.24	
0.139	0.185	75.15	7.09	3.41	14.35	Tetany.
0.109	0.184	86.79	5.81	3.17	4.23	Tetany part of period.

These experiments did not point to intoxication by ammonia as the cause of tetany. In view of the results reported by other observers, notably by MacCallum and Voegtlin,[10] by Carlson and Jacobson,[16] and by Jacobson,[17] it was considered advisable to compare the content of ammonia in the blood of normal and parathyroidectomized animals.

Three special experiments on the quantities of ammonia in the blood of normal and parathyroidectomized dogs. — In order to obtain blood for comparison from animals that were as nearly as possible in the same physiological condition, two dogs of about the same weight were used in each experiment. At 9 A. M. daily they were fed a mixture of 15 gm. of hashed meat, 5 gm. of cracker meal, 3 gm. of lard, 1 gm. of infusorial earth, and 35 c.c. of water, *per kilo*. After not less than three or four days of observation under normal conditions the parathy-

TABLE II.

URINARY DATA. SECOND EXPERIMENT

1910 April.	Weight.	Vol.	Sp. gr.	Reaction.	Nitrogen.		
					Total	Urea.	NH_3.
	kilos.	c.c.		litmus.	gm.	gm.	gm.
14	5.70	170	1018	Amphot.	2.882	2.456	0.115
15	5.70	160	1020	Amphot.	2.837	2.500	0.107
16	5.67	134	1026	Acid	3.501	2.976	0.143
17	5.73	184	1026	Acid	4.096	3.504	0.172
18	5.70	184	1023	Acid.	4.157	3.530	0.181
19	5.67	184	1025	Acid	4.193	3.690	0.163
20	5.69	184	1023	Amphot.	4.554	3.953	0.157
21	5.67	205	1022	Acid	4.521	3.882	0.244
21 P. M.	....	....	....	....	0.860	0.531	0.020[1]

[1] These figures are based upon

TABLE III.

URINARY DATA. THIRD EXPERIMENT.

1910 April.	Weight	Vol.	Sp. gr.	Reaction.	Nitrogen.			
					Total.	Urea.	NH_3.	Creatinin and creatin.
	kilos.	c.c.		litmus.	gm.	gm.	gm.	gm.
18	9.45	225	1025	Acid	5.166	4.244	0.242	0.151
19	9.45	242	1023	Acid	5.104	4.296	0.212	0.151
20	9.47	270	1021	Acid	5.181	4.448	0.242	0.112
21	9.52	240	1024	Amphot.	5.419	4.537	0.209	0.111
22	9.47	240	1025	Amphot.	5.601	4.644	0.263	0.124
23	9.47	160	1033	Amphot.	5.048	4.289	0.171	0.100
24	...	290	1026	Amphot.	6.195	5.046	0.373	0.161
24 P. M.	...	...	....	......	....	....	....	....

[1] These figures are based upon

TABLE II.

URINARY DATA SECOND EXPERIMENT.

Nitrogen.	Phosphorus in phosphate.	Percentage of total nitrogen.				
Creatinin and creatin.		Urea N.	Ammonia N.	Creatinin and creatin N.	Undetermined nitrogen.	Remarks.
gm.	gm.	per cent.	per cent.	per cent.	per cent.	
0.114	0.162	85.22	3.99	3.97	6.82	
0.132	0.169	86.02	3.76	4.65	5.57	
0.138	0.178	84.99	4.09	3.94	6.98	
0.150	....	85.55	4.21	3.67	6.57	
0.156	0.202	86.43	4.35	3.75	5.47	
0.142	0.194	88.01	3.88	3.39	4.72	
0.154	0.092	86.79	3.45	3.38	6.38	Operation 10.20 A.M. April 19.
0.153	0.123	85.87	5.39	3.39	4.35	
0.046	0.036[1]	83.16	2.35[1]	5.34	9.15	Tetany 9 A. M. to 2.40 P. M.

one determination only.

TABLE III.

URINARY DATA. THIRD EXPERIMENT.

Phosphorus.		Percentage of total nitrogen.				Remarks.
Total.	PO_4.	Urea N.	Ammonia N.	Creatinin and cr'tin N.	Undetermined nitrogen.	
gm.	gm.	per cent.	per cent.	per cent.	per cent.	
0.330[1]	0.308	82.17	4.69	2.91	10.23	......
....	....	84.17	4.16	2.95	8.72	
0.346[1]	0.304	85.85	4.68	2.17	7.30	......
0.346[1]	0.304	83.75	3.85	2.04	10.40	
0.334	0.335	82.92	4.70	2.22	10.16	......
0.248	0.241	84.98	3.39	1.99	9.64	Operation 3 P. M., April 22.
0.059	0.060	81.44	6.02	2.58	9.96	
....	....	78.01	6.00[1]	1.94	14.05	Tetany.

one determination only.

TABLE IV.

URINARY DATA. FOURTH EXPERIMENT.

June. 1910.	Weight.	Vol.	Sp. gr.	Reaction.	Acidity. c. c. of N/10 acid.	Nitrogen. Total	Nitrogen. Urea.	Nitrogen. NH_3.	Nitrogen. Creatinin.
	kilos	c. c.		litmus		gm.	gm.	gm.	gm.
1	11.45	450	1015	Acid	231	3.953	3.285	0.175	0.126
2	11.55	345	1015	Acid	215	3.018	2.605	0.094	0.105
3	11.50	405	1017	Amphot.	224	3.796	3.177	0.206	0.139
4	11.57	315	1016	Amphot.	138	2.912	2.471	0.155	0.111
5	11.55	400	1016	Acid	221	3.465	2.815	0.253	0.146
6–7 [6]	11.60	390	1024	Slightly alkaline	224 [2]	3.311	2.703	0.167	0.149
8 [7]	....	340	1017	Slightly alkaline	200	3.577	2.801	0.279	0.135
9 [8]	10.72	174	1043	Slightly alkaline	253	4.053 [3]	3.321	0.067	0.119
10 [9]	10.98	130	1047	Amphot.	210	4.374 [3]	3.600	0.166	0.102
11 [10]	10.90	245	1043	Amphot.	273	7.615 [3]	6.198	0.381	0.163
12 [11]	10.67	130 [4]	1055	Acid	259	5.631 [3]	4.488	0.469	0.111
13 [12]	10.50	210	1026	Acid	98.6	4.703	3.703	0.300	0.116
14 [13]	10.25	184	1027	Amphot.	80.2	4.463	3.508	0.260	0.118
15 [14]	9.93	190	1028	Amphot.	107	4.692	3.552	0.284	0.128
									DAILY
Fore period (June 1–5)					206	3.429	2.871	0.177	0.125
From operation to beginning of tetany (June 6–7)					224	3.311	2.703	0.167	0.149
First four days of tetany (June 8–11) . .					234	4.905	3.980	0.223	0.130
Next four-day period (June 12–15) . . .					136	4.872	3.813	0.328	0.118

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[1] One determination only. Total phosphorus of the urines of June 3, 5, and 9 was not accurately determined because only small quantities of urine were available.

[2] Per day.

[3] Measured volumes of the urines of June 9, 10, 11, 12, and 13, in volumetric flasks, were heated on a water bath, acidified, allowed to cool, made up to volume, and filtered. The filtrate was used for the determination of total nitrogen, urea, purins, etc. The residue was washed with hot water and a nitrogen determination made. The amount of albumen was calculated by multiplying the amount of nitrogen found by 6.25. The figures given above under Total Nitrogen refer to non-coagulable nitrogen only.

[4] This may be the urine of part of the period only. See the protocol of the experiment, page 111.

TABLE IV.

URINARY DATA. FOURTH EXPERIMENT.

		Albumen.	Phosphorus.		Chlorin.	Sulfur.		
Creatin.	Purin.		Total.	PO$_4$.		Total.	Total SO$_4$.	Inorg. SO$_4$.
gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.
0.007	0.0082	....	....	0.213	2.375	0.267[1]	0.176	0.145
0.011	0.0058	....	0.132[1]	0.139	1.230	0.206	0.125	0.096
0.006	0.0090	....	0.211[1]	0.197	1.212	0.203	0.186	0.122
0.006	0.0073	....	0.125[1]	0.125	1.454	0.192	0.126	0.100
0.015	0.0058	....	0.187[1]	0.173	1.900	0.230	0.155	0.130
0.027	0.0166	....	0.014	0.0145	1.152	0.326	0.227	0.194
0.030	0.0160	....	....	0.0186	2.047	0.216	0.163	0.145
0.138	0.0360	0.827	0.224[1]	0.238	0.981	0.730[1]	0.496	0.428
0.078	0.0045	0.908	0.348[1]	0.310	0.199	0.250	0.230	0.198
0.162	0.0194	2.673	0.393[1]	0.348	2.481	0.779	0.399	0.365
0.121	....	0.666	0.422[1]	0.381	0.530	0.317	0.212	0.197
0.095	....	0.120[5]	0.190	0.189	1.321	0.423	0.303	0.271
0.049	....	trace	0.211[1]	0.202	0.636	0.274	0.195	0.178
0.029	....	trace	0.213[1]	0.191	0.516	0.296	0.218	0.195
AVERAGES.								
0.009	0.0072	....	....	0.169	1.696	0.220	0.156	0.120
0.027	0.0166	....	0.014	0.145	1.152	0.326	0.227	0.194
0.102	0.0291	....	....	0.229	1.678	0.494	0.322	0.284
0.074	....	....	0.259	0.241	0.751	0.328	0.232	0.210

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[5] Determined by the Esbach method.

[6] Parathyroidectomy, June 5, 11 A. M.

[7] Mild tetany.

[8] Marked tetany part of day. Urine contained 0.475% of albumen.

[9] No tetany. Urine contained 0.698% of albumen.

[10] Tetany. Bile pigments and cellular detritus in urine containing 1.09% of albumen.

[11] Mild tetany. Bile pigments, cellular detritus and spermatozoa in urine containing 0.51% of albumen.

[12] Violent tetany. No bile pigments in urine.

[13] Mild tetany. First fasting day.

[14] Marked tetany. Second fasting day.

TABLE V.

DISTRIBUTION OF NITROGEN AND SULFUR.[1] FOURTH EXPERIMENT.

1910 June.	Percentage of total nitrogen.						Sulfur percentages.		
							Total sulfur.		Total sulfate.
	Urea nitrogen.	Ammonia nitrogen.	Creatinin nitrogen.	Creatin nitrogen.	Purin nitrogen.	Undetermined nitrogen.	Sulfate sulfur.	Inorg. sulfate sulfur.	Inorganic sulfate.
	per cent.	per cent.	per cent.	per cent.	per cent.	per cent.	per cent.	per cent.	per cent.
1	83.12	4.42	3.19	0.17	0.26	8.84	65.82	54.29	82.54
2	86.02	3.17	3.47	0.37	0.19	6.78	60.66	46.69	76.06
3	83.65	5.43	3.65	0.17	0.24	6.86	91.64	60.34	65.85
4	84.93	5.33	3.81	0.21	0.25	5.47	65.45	51.95	79.38
5	81.22	7.31	4.22	0.41	0.17	6.67	67.32	56.33	83.70
6–7	81.67	5.03	4.50	0.80	0.50	7.50	69.61	59.61	85.63
8	78.30	7.80	3.78	0.83	0.45	8.84	75.23	66.91	88.94
9	81.92	1.66	2.94	3.41	0.89	9.18	67.97	58.60	86.22
10	82.31	3.79	2.33	1.78	0.10	9.69	92.06	79.34	86.18
11	81.38	5.00	2.15	2.12	0.25	9.10	51.17	46.58	91.01
12	79.70	8.32	1.97	2.14	....	7.87 [2]	66.97	61.98	92.55
13	78.73	6.38	2.46	2.03	....	10.40 [2]	71.58	64.18	89.66
14	78.61	5.82	2.63	1.11	....	11.83 [2]	71.12	64.85	91.33
15	77.81	6.06	2.72	0.63	....	12.78 [2]	73.77	65.85	89.23
DAILY AVERAGES.									
1–5 [3]	83.79	5.13	3.49	0.26	0.22	7.11	70.18	53.92	77.51
6–7 [4]	81.67	5.03	4.50	0.80	0.50	7.50	69.61	59.61	85.63
8–11 [5]	80.98	4.51	2.80	2.04	0.65	9.18	71.61	62.86	88.09
12–15 [6]	78.73	6.64	2.45	1.49	....	13.22 [2]	70.86	64.22	90.69

[1] See remarks in Table IV.

[2] Includes purin nitrogen.

[3] Fore period.

[4] From operation to beginning of tetany.

[5] First four days of tetany.

[6] Second four days of tetany.

roids were removed from one dog. Shortly after tetany appeared, the dog was anesthetized with ether and bled from a carotid artery. Two or three samples of blood were run into weighed flasks containing 5 c.c. of a 10 per cent solution of potassium oxalate. The dog was then exsanguinated, the rest of the blood being preserved for use in other work. The above-mentioned samples of oxalated blood were then transferred to an apparatus for the determination of ammonia by Folin's method.[30] The period of aeration was eight hours. One-hundredth normal solutions of sulfuric acid and sodium hydroxide were used for the titrations. Iodeosin in ethereal solution was the indicator. The control dog was bled at the same hour a few days later. Table X summarizes the results obtained.

The results obtained from a given sample of blood did not always agree very closely. It is quite evident, however, that in these experiments the blood of dogs in tetania parathyropriva did not contain more ammonia than the quantity present in normal dog blood obtained under similar conditions.

Urea. — The percentage of nitrogen excreted as urea fell considerably after the operations. In one case (first experiment) it again rose to the normal but, as was explained above, this dog had apparently recovered from tetany.

Purins. — The purin excretion in the one experiment in which it was studied (fourth experiment) was greater after the operation than before. This increase occurred prior to the appearance of tetany and was followed by a further increase. Probably some, at least, of this augmented purin output was due to the muscular work involved in tetany, for Burian[31] has shown that severe muscular work increases the elimination of purin substances.

Creatinin. — The creatinin excretion in the first three experiments showed no noteworthy changes other than an increased output, both absolutely and relatively to the total nitrogen, after the appearance of tetany in the second experiment. The fourth experiment, in which both creatin and creatinin were determined and in which the dog survived for a comparatively long period, yielded rather striking results. In the urine of the two-day period following the operation both creatinin and creatin were increased, but after the onset of tetany the

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[30] Folin: Zeitschrift für physiologische Chemie, 1902, xxxvii, p. 161.

[31] Burian: Zeitschrift für physiologische Chemie, 1905, xliii, p. 533.

TABLE VI.

DATA PERTAINING TO THE EXCRETION OF NITROGEN, AMMONIA. AND PHOSPHORUS. FIFTH EXPERIMENT.

1910. Nov.	Weight.	Urine.								Feces[1] (dry).	
		Vol.	Spec. gr.	Reaction.	Tot. N.	NH_3-N.	$\frac{NH_3\text{-}N.}{Tot. N.}$	Phosphorus.		Weight	Phosphorus per gram.
								Total.	PO_4.		
	kilos.			litmus.	gm.	gm.	per cent.	gm.	gm.	gm.	mg.
27	9.50	165	1021	Acid	3.993	0.209	5.23	0.215	0.213	21.5	3.16
28	9.40	245	1021	Acid	5.051	0.215	4.26	0.292	0.299	18.6	3.14
29	9.40	160	1032	Acid	4.801	0.165	3.44	0.339	0.322	13.9	3.59
30	9.40	190	1030	Acid	5.141	0.208	4.04	0.406	0.388	16.3	3.69
Dec.1	9.35	208	1025	Acid	4.680	0.235	5.02	0.377	0.320	19.8	3.37
2	9.35	215	1021	Acid	4.262	0.197	4.63	0.343	0.331	22.7	3.00
3	9.25	220	1019	Acid	4.275	0.229	5.34	0.307	0.259	23.7	2.84
4	9.20	175	1025	Acid	4.175	0.204	6.16	0.257	0.207	18.1	2.94
5	9.10	238	1017	Acid	4.113	0.171	4.15	0.270	0.270	27.6	2.98
6[2]	9.25	160	1022	Neutral	4.047	0.135	3.34	0.029	0.028	4.6	3.99[3]
7	9.10	290	1013	Acid	4.033	0.178	4.41	0.028	0.026	21.2	2.80
8[4]	9.00	250	1017	Slightly acid	4.035	0.213	5.28	0.064	0.052	16.3	2.93
9[5]	8.90	225	1023	Slightly acid	4.318	0.234	5.41	0.254	0.230	8.2	3.05
10[6]	8.70	105	1038	Acid	2.565	0.227	8.83	0.210	0.196	16.0	2.83

[1] Since the weight of the daily feces varied greatly and since the infusorial earth given with the food constituted the greater part of the feces analyzed, it seemed best not to calculate the total daily excretion of phosphorus in the feces, but to express the results in terms of milligrams of phosphorus per gram of dry feces.

[2] Parathyroids removed at 11 A. M. December 5.

[6] No food.

TABLE VII.

Data pertaining to the Excretion of Nitrogen, Ammonia, and Phosphorus. Sixth Experiment.

1910. Jan.	Weight.	Urine.								Feces[1] (dry).	
		Vol.	Spec. gr.	Reaction.	Tot. N.	NH_3–N.	$\frac{NH_3\text{–N.}}{\text{Tot. N.}}$	Phosphorus.		Weight	Phosphorus per gram.
								Total.	PO_4.		
	kilos.			litmus.	gm.	gm.	per cent.	gm.	gm.	gm.	mg.
14	10.62	160	1035	Acid	5.211	0.258	4.95	0.420	0.403	15.0	3.61
15	10.64	225	1030	Acid	5.540	0.292	5.27	0.426	0.422	22.6	3.62
16[2]	10.67	....	....	....	....	....	....	....	....	24.3	3.89
17	10.70	260	1023	Acid	5.554	0.308	5.54	0.398	0.366	23.7	3.68
18	10.60	265	1023	Acid	5.504	0.323	5.87	0.447	0.401	26.2	3.63
19	10.57	260	1021	Acid	4.811	0.392	8.15	0.303	0.301	22.9	3.23
20[3]	10.59	260	1027	Acid	5.584	0.245	4.39	0.110	0.098	20.5	3.54
20 P.M.[4]	....	125	1017	Acid	2.724	0.146	5.34	0.066	0.058	10.7	3.63

[1] Since the weight of the daily feces varied greatly, and since the infusorial earth given with the food constituted the greater part of the feces analyzed, it seemed best not to calculate the total daily excretion of phosphorus in the feces, but to express the results in terms of milligrams of phosphorus per gram of dry feces.

[2] Urine lost by accident.

[3] Parathyroidectomy at 10 A. M., January 19.

[4] Tetany began at 3 P. M., January 20. The dog was bled to death, under ether anesthesia, at 3.45 P. M. Urine obtained from bladder, and feces from large intestine.

absolute output of creatinin was decreased, while that of creatin rose rapidly and exceeded that of creatinin. On the last four days the creatin output decreased while the creatinin excretion remained about the same.

Undetermined nitrogen. — As a result of the changes in the excretion of urea, ammonia, creatinin, and creatin, the ratio of the "undetermined nitrogen" to the total nitrogen rose markedly with the appearance of tetany. In the first experiment it dropped below the normal on the third day after the operation. As has been explained above, this is possibly connected with the dog's recovery from tetany.

TABLE VIII.

RATIO OF AMMONIA NITROGEN TO TOTAL NITROGEN.

Exp.	Days before the operation.								
	9	8	7	6	5	4	3	2	1
	per cent.	per cent.	per cent.	per cent.	per cent.	per cent.	per cent.	per cent.	per cent.
1	...	...	...	...	...	5.77	5.46	5.94	4.22
2	...	...	...	3.99	3.76	4.09	4.21	4.35	3.88
3	...	...	...	...	4.69	4.16	4.68	3.85	4.70
4	...	...	...	...	4.42	3.17	5.43	5.33	7.31
5	5.23	4.26	3.44	4.04	5.02	4.63	5.34	6.16	4.15
6	...	...	...	4.95	5.27	...	5.54	5.87	8.15
Average	...	...	...	...	...	...	5.11	5.25	5.40

[1] This figure pertains to urine of only six hours and is based upon only one determination.

[2] This figure pertains to urine of only nine hours.

Chlorids. — The excretion of chlorids after parathyroidectomy was very irregular. Probably the loss of hydrochloric acid with the vomit had considerable influence in this connection. However, the lowered output in the two-day period following the operation cannot be explained in this manner, for the volume of vomit was entirely too small.

Sulfur. — The excretion of sulfur varied so greatly from day to day that an inspection of the daily records discloses nothing characteristic. But, if the averages for the various periods are examined, we see that immediately after the removal of the parathyroids the total sulfur output rose very markedly. The increase was entirely in the forms of inorganic sulfate and neutral sulfur; the excretion of ethereal sulfate remained almost unchanged. The increase in the elimination of inorganic sulfate was proportionally greater than the rise in the total sulfur excretion, the ratio of inorganic sulfate sulfur to total sulfur changing from 53.92 to 62.86 per cent. Beginning with the seventh day after the operation, the sulfur and sulfate excretions fell considerably. The decrease in the amount of inorganic sulfate was not quite so marked as that of the total sulfur, and consequently the ratio of inorganic sulfate to total sulfur rose. The excretion of ethereal sulfate decreased both absolutely and relatively.

TABLE VIII.

Ratio of Ammonia Nitrogen to Total Nitrogen.

Day of parathyroidectomy.	Days after the operation.								
	1	2 Tetany began.	3	4	5	6	7	8	9
per cent.	per cent.	per cent.	per cent.	per cent.	per cent.	per cent.	per cent.	per cent.	per cent
4.35	4.53	7.09	5.81	...	...	...	...	...	...
3.45	5.39	2.35[1]	...	...	...	...	...	...	...
3.39	6.02	6.00[2]	...	...	...	...	...	...	...
5.03	5.03	7.80	1.66	3.79	5.00	8.32	6.38	5.82	6.06
3.34	4.41	5.28	5.41	8.83[3]	...	...	...	...	...
4.39	5.34[4]	...	...	...	...	...	...	...	...
3.99	5.12	5.70	...	...	...	...	...	...	...

[3] No food on this day.

[4] Tetany appeared on the day following the operation. Urine of only six hours obtained.

Beck and Benedict,[32] Munk,[33] and Dunlap and collaborators [34] found that severe muscular work is followed by a very considerable increase in the output of inorganic sulfates, a less marked rise in the elimination of neutral sulfur, and absolutely no change in the ethereal sulfate excretion. Apparently, then, the changes observed in the sulfur excretion on the six days immediately following the operation may be

TABLE IX.

Excretion of Phosphorus in the Urine.

Exp.	Form of phosphorus determined.	Method.[1]	Days before the operation.							
			9	8	7	6	5	4	3	2
			gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.
1	Phosphate	Uran. acetate	..	..	..	..	..	0.177	0.183	0.160
2	Phosphate	Uran. acetate	..	..	..	0.162	0.169	0.178	..	0.202
3 [3]	Phosphate	Uran. acetate	..	..	..	..	0.308	..	0.304	0.304
3	Total	Liebig	..	..	..	..	0.330	..	0.346	0.346
4	Phosphate	Uran. acetate	..	..	..	..	0.213	0.139	0.197	0.125
4 [4]	Total	Liebig	..	..	..	..	..	0.132	0.211	0.125
5	Phosphate	Mathison	0.213	0.299	0.322	0.388	0.320	0.331	0.259	0.207
5	Total	Neuman	0.215	0.292	0.339	0.406	0.377	0.343	0.307	0.257
6	Phosphate	Mathison	..	..	..	0.403	0.422	..	0.366	0.401
6	Total	Neuman	..	..	..	0.420	0.426	..	0.398	0.447
Control [5]	Phosphate	Uran. acetate	..	..	..	0.159	0.233	0.167	0.143	0.172[6]

[1] See page 108.

[2] This is about one fifth of the twenty-four-hour excretion (estimated from the nitrogen excretion). One determination only.

[3] This dog was operated upon at 3 P. M., after having been fed at 9 A. M.

[4] Most of these figures are based upon one determination only. See footnote to Table IV.

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[32] Beck and Benedict: Archiv für die gesammte Physiologie, 1893, liv, p. 27.

[33] Munk: Archiv für Physiologie, 1895, p. 385.

[34] Dunlap, Paton, Stockman, and Maccadam: Journal of physiology, 1897, xxii, p. 68.

due largely to the excessive muscular activity involved in tetany. It is significant that during the period ending April 10, when tetany was absent, the sulfur excretion was nearly normal. The changes that occurred toward the close of the experiment may have been due to the lack of food, for Benedict[35] found that the total sulfur output, and the proportion excreted as neutral sulfur, may be diminished in inanition. However, the increased sulfur excretion before the onset of tetany is still to be explained.

TABLE IX.

EXCRETION OF PHOSPHORUS IN THE URINE.

	Day of para-thyroi-decto'y.	Days after the operation.								
1		1	2 Tetany began.	3	4	5	6	7	8	9
gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.
0.175	0.110	0.086	0.185	0.184	...	..	..	..	..	..
0.194	0.092	0.123	0.036 [2]	....	...	..	..	..	..	..
0.335	0.241	0.060	...	...	...	...	..	..	..	..
0.334	0.248	0.059	...	...	...	...	..	..	..	..
0.173	0.0145[6]	0.0145[6]	0.0186	0.238	0.310	0.348	0.381	0.189	0.202	0.191
0.187	0.014 [6]	0.014 [6]	...	0.224	0.348	0.393	0.422	0.190	0.211	0.213
0.270	0.028	0.026	0.052	0.230	0.196	...	..	..	..	..
0.270	0.029	0 028	0.064	0.254	0.210	...	..	..	..	..
0.301	0.098	0.058 [7]	...	...	...	...	..	..	..	..
0.303	0.110	0.066 [7]	...	...	...	...	..	..	..	..
0.172 [6]	0.162	0.175	...	...	...	...	..	..	..	..

[5] The dog was anesthetized; the glands exposed, handled, etc., but not removed.

[6] Determinations made in the forty-eight hour sample.

[7] Tetany appeared on the day following parathyroidectomy. The figures for this day pertain to only a fraction of the twenty-four-hour urine. See remarks in Table VI.

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[35] BENEDICT: Influence of inanition on metabolism, Washington, 1907, p. 397.

Phosphorus. — By far the most remarkable observation in these experiments was the very great decrease in the elimination of phosphate after parathyroidectomy. This was noted in each of the six experiments, the phosphate excretion falling to not more than 50 per cent, and to as little as 8 per cent of its former amount. This decrease was not accompanied by an increased output of organic phosphorus in the urine nor by an increased excretion of phosphorus in the feces. The fact that with the appearance of tetany, or soon thereafter, the phosphorus excretion rose to the normal suggested the possibility that the diminished phosphorus output was due to the anesthetic or other factors incidental to the operation. Accordingly a normal dog was anesthetized on the morning of July 18, 1910, and the operation performed in the usual manner, except that the parathyroids were not removed. Ether was administered for one hour, the maximum time

TABLE X.

Quantities of Ammonia in the Blood of Normal and Parathyroidectomized Dogs.

Exp.	Dogs in Tetany.				Controls.	
	Time since operation.	Time since last meal.	Time since tetany was first observed.	Ammonia in 100 gm. of blood.	Time since last meal.	Ammonia in 100 gm. of blood.
	hours.	hours.	hours.	mg.	hours.	mg.
I	30	6	1.5	0.660 0.816	6	1.297 1.311 1.314
II	72	6	1.0	0.538 0.859	6[1]	1.000 0.984 0.564
III	50	7	2	0.676 0.692 0.724	7	0.905 0.973 0.873

[1] Ten days after parathyroidectomy; no tetany.

required for any of the previous operations. The phosphate excretion in this dog did not change after the operation.[36] Apparently then the diminished phosphorus output after parathyroidectomy is related to the parathyroid insufficiency.

Engelman,[37] Klug and Olsavsky,[38] and Paton,[34] found that excessive muscular work, particularly if the subject was out of training, was followed by an increased excretion of phosphoric acid. Klug and Olsavsky [38] found that a dog weighing 5.25 kilos excreted, in a ten-day control period, an average of 0.3175 gm. of P_2O_5 per day. The minimum was 0.24 gm.; the maximum was 0.39 gm. The urine of the work-day, upon which the dog ran 16 kilometres, contained 0.57 gm. of P_2O_5. The excretion on the next day, which was spent in the cage, was 0.28 gm. Muscular work, then, was followed by an increase of 0.25 gm. of P_2O_5 in the urine. In a dog weighing 11 kilos the increase, at the same ratio, would be 0.53 gm. of P_2O_5 or 0.23 gm. of P. As the work involved in tetany is much more severe and exhausting than that performed by the dog in Klug and Olsavsky's experiment, it is quite probable that the greater part of the phosphorus excreted after parathyroidectomy by the dog in the fourth experiment (in which the increased excretion of phosphorus after the appearance of tetany was most marked), was caused by the tetany. This would imply that the tendency to phosphorus retention, which was so evident on the first three days of that experiment, persisted until the death of the animal. That the phosphate excretion was not increased on April 7–8 nor diminished on April 9–10 (fourth experiment) does not conflict with this view, for Paton [33] found that after unusual muscular work the excretion of phosphorus is not increased on the work-day but on the two days following.

V. Summary of Results.

The following results were obtained in the experiments on parathyroidectomized dogs:

1. The excretion of nitrogen was increased only after the appearance of tetany.

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[36] A similar result was obtained in another connection in this laboratory some years ago. See Hawk and Gies: This journal, 1904, xi, p. 217.

[37] Engelman: Archiv für Anatomie und Physiologie, 1871, p. 14.

[38] Klug and Olsavsky: Archiv für die gesammte Physiologie, 1893, liv, p. 21.

2. The ratio of urea nitrogen to total nitrogen was decreased.

3. The proportion of the total urinary nitrogen excreted in the form of ammonia was increased very little, if at all.

4. The concentration of ammonia in the blood was not higher than in the blood of normal animals.

5. The excretion of creatinin remained about the same as before operation, or was slightly diminished.

6. The creatin of the urine was very much increased.

7. The purin nitrogen of the urine was increased.

8. There was an increased elimination in the urine of nitrogenous constituents of unknown nature ("undetermined nitrogen").

9. The excretion of sulfur in the urine was augmented, the increase being chiefly in the forms of inorganic sulfate and neutral sulfur.

10. There was a very marked phosphorus retention on the first few days after the removal of the parathyroids. This was followed, but not until after the appearance of tetany, by an increased excretion of phosphorus.

VI. Conclusions.

The increased excretion of nitrogenous compounds of unknown nature after parathyroidectomy may be due to diminished activity of the liver. However, the experiments here described do not lend support to the view that the tetany following parathyroidectomy is due to intoxication by ammonia or by carbamic acid.

Since the diminished excretion of phosphorus is the most striking change in the metabolism as yet observed after parathyroidectomy, it is probable that an investigation of the form in which the phosphorus is retained will throw some light on the function of the parathyroid gland. It is my intention to make such a study in the near future.

It is a pleasure to record here my indebtedness and gratitude to Prof. W. G. MacCallum and to Prof. William J. Gies for the invaluable help and advice they freely gave me.

ADDENDUM.

Several days after the foregoing had been sent to the editor, Cooke's[39] paper, on the "Changes in nitrogenous metabolism after parathyroidectomy," appeared. Cooke's results may be summarized as follows:

1. After parathyroidectomy and partial thyroidectomy the excretion of nitrogen and ammonia was increased. The ammonia ratio was higher than before the operation.
2. The ratio of urea nitrogen to total nitrogen was diminished to a greater extent than was accounted for by the increased ammonia excretion.
3. The excretion of creatinin remained almost unchanged, but the percentage of total nitrogen as creatinin was markedly diminished.
4. The amino-nitrogen (determined by Van Slyke's method) changed with the total nitrogen.
5. In the two experiments in which the urines were examined for the presence of lactic acid, it was found that this substance appeared coincident with the appearance of tetany.

These findings accord, in part, with those obtained by the author. Very marked differences, however, exist between Cooke's results and the author's for the excretion of ammonia. In Cooke's experiments the ammonia ratio was increased, in the author's experiments it was not. This disagreement is probably due to the differences in the experimental procedures. The fact that Cooke used partially thyroidectomized animals is of comparatively little significance, for Underhill and Hilditch[40] found a normal ammonia ratio in the urines of thyroidectomized dogs retaining at least two parathyroids.

Cooke used fasting animals in all but one of his experiments. The author kept all his dogs upon a fixed diet. It is possible that the reaction of the fasting organism to removal of the parathyroids may be somewhat different from that of a well-fed animal. In the fasting animal it is more probable that a certain degree of acidosis would ensue. Cooke seems to think that the high ammonia ratios were due to an increased formation, or diminished destruction, of organic acids,

[39] Cooke: Journal of experimental medicine, 1911, xiii, p. 439.

[40] Underhill and Hilditch: This journal, 1909, xxv, p. 66.

such as lactic acid. The fact that the urines obtained after parathyroidectomy were frequently alkaline indicates that this is not, in itself, a sufficient explanation.

Another important point of difference was that Cooke's animals were catheterized daily, whereas the author's were not. Cooke states that cystitis did not develop in any of his dogs. However the absence of a perceptible cystitis is not proof that there was not a slight infection which was sufficient to increase the ammonia ratio to the extent observed. Some of Cooke's results point in this direction. In one experiment in which tetany did not develop, the ammonia of the urine was greatly increased. Again, in another experiment in which marked tetany did appear, the ammonia ratio was not at all increased. There is, therefore, nothing in Cooke's results which is at real variance with the conclusions stated above.

FURTHER STUDIES ON THE NATURE OF PARATHYROID TETANY.

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THE work here reported was undertaken mainly with the view of further testing the hypothesis that the ammonia in the blood is a factor in the excitation phenomena following parathyroidectomy. Previous work seemed to confirm the findings of MacCallum and Voegtlin[1] of increased ammonia in the blood of animals in parathyroid tetany.[2] There seems to be no doubt of the absolute and relative increase of ammonia in the urine in parathyroid tetany. This fact would demand some increase in the blood ammonia, but this increase may, of course, be too slight to be determined by our present methods. The similarity of the symptoms to those of meat poisoning in Eck fistula dogs, and the fact that the symptoms seem to be intensified by meat feeding, suggested that the removal of these glands leads, directly or indirectly, to depression of the liver and consequent appearance of toxic products of protein metabolism in the blood. Some work already reported points to depression of the liver functions, but before proceeding further in the study of the liver activity in parathyroidectomized animals, it seemed necessary to make a more detailed comparative study of ammonia and parathyroid tetany. Notwithstanding the difficulty in differentiating between primary and secondary factors in the symptom complex, and elements of uncertainty in conclusions as to curative measures, because of the periodicity of the symptoms, our present results indicate that the disturbance following parathyroidectomy involves the digestive tract and the circulatory system, and that none of the curative measures (save transplantation) that have been so far found to be temporary palliatives have *specific* significance.

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[1] MacCallum and Voegtlin: Journal of experimental medicine, 1909, xi, p. 118.

[2] Carlson and Jacobson: This journal, 1910, xxv, p. 403; xxvi, p. 407.

I. The Influence of Calcium Salts on the Ammonia Content of the Blood.

1. **The action of calcium salts in parathyroid tetany.** — The great amount of experimental work on the action of calcium salts on animal tissues, normal as well as pathological, indicates a general depressor action. The vertebrate heart is a possible exception, and other exceptions are recorded from time to time in the case of tissues rendered abnormal by the addition or withdrawal of other salts. Of particular interest in this connection are the observations of Sabbatani,[3] Roncoroni,[4] and MacCallum and Voegtlin.[5] Roncoroni showed that the reduction of calcium in the circulating blood of the brain by the use of a substance supposedly non-toxic in itself, increases the excitability of the motor zone of the cortex, provoking epileptiform phenomena, and that these in turn are suppressed by adding calcium to the circulating fluid. MacCallum and Voegtlin showed that it is possible to temporarily suppress the symptoms following parathyroidectomy by injecting calcium salts. Berkeley and Beebe [6] confirmed these results, and in addition found that salts of the chemically closely allied substance, strontium, had a similar and equally pronounced effect. Biedl [7] reports similar results, but he does not find that these salts prolong the life, despite the suppression of the excitation symptoms.

Our present series of experiments were made on dogs. Both thyroids and parathyroids were removed. Calcium lactate in 5 per cent concentration was used, and the injections made intravenously in all cases. The following protocols illustrate the dependence of the action on the relative severity of the tetany symptoms:

Experiment No. 3. — Medium-sized dog.

Nov. 29.	Complete thyroid-parathyroidectomy.
Dec. 1, 8.30 A. M.	Tetany, tremors in all muscles; snapping of jaws; rapid heart and respiration.

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[3] Sabbatani: Rivista sperimentale di Feniatria, 1901, xxviii, p. 946.

[4] Roncoroni: Archives italiennes de biologie, xlii, p. 472.

[5] MacCallum and Voegtlin: *Loc. cit.*

[6] Berkeley and Beebe: Journal of medical research, 1909, xx, p. 149.

[7] Biedl: Innere Sekretion, Berlin, 1910.

9.22 A. M.	10 c.c. Ca; heart slower; respiration slower; tetany and tremors diminished.
9.40 A. M.	10 c.c. Ca; complete cessation of tetany and disappearance of tremors; depressed and sleepy.
3.30–5.00 P. M.	Few transitory spasmodic contractions of shoulder muscles and slight tremors at intervals, respiration slow and deep, strong diaphragm contractions with each heart beat.
Dec. 2, 3.00 P. M.	Tetany; tremors; salivation.
3.10 P. M.	10 c.c. Ca injected.
3.35 P. M.	10 c.c. Ca injected.
	Between these two injections and following them, there were several convulsions; tremors and salivation were still present. 4 P. M. the dog died in a violent tetanic convulsion with respiratory standstill, artificial respiration proving of no avail.

Experiment No. 6. — Dog; weight, 8 K.

Dec. 6.	Complete thyroid-parathyroidectomy.
Dec. 7.	In good condition.
Dec. 8, 4.00 P. M.	Rapid respiration; salivation; tremors in all muscles.
8.00 P. M.	Vomiting; rapid respiration; salivation; tremors. 20 Ca injected; symptoms disappeared.
Dec. 9, 5.00 P. M.	Depression and slight tremors.
Dec. 10, 8.30 A. M.	Tremors; 20 c.c. Ca; tremors ceased.
8.30 P. M.	Slight tetany, tremors and irregular respiration; 20 c.c. Ca; tremors ceased.
Dec. 11, 9.30 A. M.	Tremors pronounced; respiration rapid; weak, staggering gait.
12.30 P. M.	14 c.c. Ca; tremors ceased.
8.30 P. M.	Extreme depression; tremors; 20 c.c. Ca; tremors immediately disappeared; depression noticeably less profound.
Dec. 12, 8.30 A. M.	Tremors; irregular respiration; depression; 20 c.c. Ca; cessation of symptoms.
12.30 P. M.	Quite normal; ate quantity of meat.
2.00 P. M.	Tremors and dyspnœa; 14 c.c. Ca; cessation of symptoms.
Dec. 13, 8.30 P. M.	Tetany and tremors; 20 c.c. Ca; symptoms less severe.

Dec. 14, 11.45 A. M. Profound depression, irregular respiration, tremors; 20 c.c. Ca; tremors persisting.

12.30 P. M. Animal died in convulsions, but not extreme tetany.

Experiment No. 8. — Medium-sized dog.

Dec. 5, 3.00 P. M. Complete thyroid-parathyroidectomy.

Dec. 6, 8.30 A. M. Apparently normal condition.

11.30 P. M. Respiration irregular and labored; strong muscular tremors. 18 c.c. Ca injected, followed by normal respiration, slow heart, and entire relief from the muscular symptoms in five minutes.

Dec. 7, 3.40 P. M. Tetanic contractions of fore and hind leg muscles.

4.25 P. M. 15 c.c. Ca injected, complete and immediate cessation of symptoms.

Dec. 8, 9.00 A. M. Violent tetany; 15 c.c. Ca; symptoms ceased and animal ate some meat.

2.00 P. M. Tremors present and respiration very rapid.

3.00 P. M. 20 c.c. Ca injected; respiration slower; tremors disappeared within five minutes.

Dec. 8, 9.30 P. M. 15 c.c. Ca injected as prophylactic.

Dec. 9, 8.30 A. M. Tetany; strong contractions of extensors.

11.45 A. M. 20 c.c. Ca; prompt suppression of tremors.

5.00 P. M. Slight tremors, 20 c.c. Ca; cessation of symptoms.

Dec. 10, 8.30 A. M. Irregular respiration and tremors; 20 c.c. Ca; tremors ceased.

12.30 A. M. Irregular respiration; 15 c.c. Ca; tremors ceased.

8.30 P. M. Slight tremors; 20 c.c. Ca; tremors ceased.

Dec. 11, 9.30 A. M. Severe tetany; tremors; snapping of jaws; dyspnœa. 15 c.c. Ca; gradual cessation of the symptoms.

5.30–8.30 P. M. Intermittent violent tetanic spasms with respiratory standstill, tremors pronounced; animal apparently very weak. 20 c.c. Ca; immediate and striking relief.

Dec. 12, 8.30 A. M. Tremors; tetany and convulsions with respiratory standstill; rapid and irregular respiration, 20 c.c. Ca; gradual cessation of symptoms.

2.00 P. M. Tremors and dyspnœa.

8.00 P. M. Slight tremors, great depression. 15 c.c. Ca; some improvement.

Dec. 13, 8.30 A. M. Animal found dead.

It seems, from these experiments and others in this series, that in the later stages of the parathyroid tetany calcium in larger and more frequent doses is necessary to suppress the symptoms, and that finally even large quantities of calcium salts introduced into the circulation will not prevent death in convulsion or tetany. The fact remains, however, that in the initial and in some very acute stages of parathyroid tetany the calcium salts do temporarily suppress the symptoms, and that in this manner the life of the animal is undoubtedly prolonged.

2. The ammonia content of the blood in parathyroid tetany before and after calcium injection. — In previous work an increased amount of ammonia has been found in the blood of animals in parathyroid tetany. This led us to the working hypothesis that the symptoms are in part due to ammonia intoxication. If this is the case, the calcium may act (1) by increasing the elimination of ammonia; (2) by indirect neutralization, or (3) by depression of the tissue. Muirhead and Abel[8] found that calcium injected subcutaneously or intravenously is excreted as calcium carbamate. The close chemical relationship between calcium carbamate and ammonium compounds (especially the carbonate) suggests the possibility of the conversion of excess of ammonium carbonate or carbamate into calcium carbamate which may be less toxic or more readily excreted than are the ammonium salts. On the other hand, the calcium salts may directly stimulate the kidney cells to eliminate ammonium salts. This does not seem probable in view of the fact that an increased calcium content of the blood tends to diminish the secretion of urine. The calcium may detoxicate the blood indirectly by stimulation of the liver. In any of these cases a blood analysis should show a decreased ammonia content. To test the hypothesis, blood samples were drawn from dogs in tetany and again immediately after the symptoms had been suppressed by calcium injections. In the first part of the work the analyses were made according to the old method of Folin (using *n*/50 solutions for titrations instead of the *n*/20 he advises). The results of these analyses are given in Table I. These experiments were repeated using Nessler's reagent instead of titrating, to determine the amount of ammonia recovered. The results of this series are given in Table II, together with results from samples of normal blood analyzed by this method. It seems clear from these experiments *that calcium does not appreciably decrease the amount of ammonia in the blood in parathyroid tetany, even though it completely suppresses the excitation symptoms.*

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[8] Muirhead and Abel: Archiv für experimentelle Pathologie und Pharmacologie, 1892, xxxi, p. 15.

TABLE I.

AMMONIA CONTENT OF THE BLOOD AS DETERMINED BY FOLIN'S METHOD.

Normal Dogs.		Parathyroidectomized Dogs		
No.	Mgr. NH_3 per 100 c.c.	No.	Mgr. NH_3 per 100 c.c. blood in animals	
			with tetany symptoms.	whose symptoms have been suppressed by Ca.
1	1.36	1	1.93	1.30
2	1.52	2	2.02	2.12
3	1.42	3*a*	2.66	2.59
4	1.38	3*b*	2.94	2.80
5	1.63	3*c*	2.44	2.97
6	1.42	3*d*	1.84	1.90
7	1.22	3*e*	2.29	2.10
8	1.37	4*a*	1.66	1.75
9	1.45	4*b*	1.90	2.14
10	1.32	4*c*	2.19	1.90
..	....	5*a*	2.24	1.63
..	....	5*b*	1.66	1.27
..	....	5*c*	1.67	1.36
..	....	5*d*	1.71	1.98

The action of calcium must therefore be explained on some other basis than the diminution of the blood ammonia (assuming that the methods of the ammonia determinations are sufficiently accurate).

The second series shows a lower ammonia content in the blood of both the normal and the tetany animals. This would indicate a difference in the accuracy of the two methods. Since the Nessler method appears to be the more delicate one for small quantities of ammonia, some control experiments were carried out to determine whether, in our hands, a slight increase in the ammonia in the blood can be estimated correctly. Normal dogs were bled sufficiently for two analyses. To 25 c.c. of the blood a carefully measured quantity of ammonia (in the form of chloride) (0.1 to 0.2 mg. NH_3) was added and the amount recovered calculated by subtracting the quantity found in the control. The results of these experiments showed the quantity

TABLE II.

AMMONIA CONTENT OF THE BLOOD AS DETERMINED BY THE NESSLER METHOD.

NORMAL DOGS.		PARATHYROIDECTOMIZED DOGS.		
No.	Mgr. NH_3 per 100 c.c.	No.	Mgr. NH_3 per 100 c.c. blood in animals	
			with tetany symptoms	whose symptoms have been suppressed by Ca.
1	0.81	1*a*	0.60	0.60
2	0.81	1*b*	0.93	1.00
3	0.65	1*c*	0.92	0.85
4	0.74	1*d*	1.06	0.95
5	0.83	1*e*	1.40	1.14
6	0.60	1*f*	1.30	.90
7	0.73	1*g*	0.90	0.73
8	0.88	2	1.33	1.25
9	1.09	3	0.95	1.00
10	0.70	4*a*	0.92	1.00
11	1.10	4*b*	0.60	0.60
..	....	5	1.20	no analysis
		6*a*	0.90	" "
..	....	6*b*	0.80	" "
..	....	6*c*	0.60	" "

recovered to be correct within 10 per cent of the quantity added (the error not exceeding .02 mg.), thus indicating that the ammonia present is recovered and estimated quantitatively within a reasonable degree of accuracy. Slight dilution of the blood with water, to render the solution less viscous, seems to have no appreciable effect on the liberation of the ammonia. Results calculated for 100 c.c., using 10 c.c. blood, were slightly higher than when 40 c.c. were used. This is in accordance with Folin's observations.

It will be noted that the *analysis with the Nessler reagent does not show any marked or constant increase in the ammonia content of the blood in parathyroid tetany as compared with that of the blood from normal dogs.* Contrary to the previous results of MacCallum and Voegtlin and ourselves, using the titration method. We are at a loss to account for the difference unless it is due to the difficulty of estimating small quantities of ammonia by titration. There is more than the normal amount of ammonia produced or less ammonia destroyed in the animal in parathyroid tetany, but the activity of the kidneys apparently prevents an appreciable accumulation of the ammonia in the blood above that in the normal animal. This rapid elimination of excess ammonia is in accordance with the findings in certain clinical cases of deranged metabolism with acidosis, large quantities of ammonia (1 to 12 gm.) being eliminated in twenty-four hours, without any increase in the blood ammonia.

II. PARATHYROID TETANY VERSUS AMMONIA TETANY.

1. **Comparison of symptoms.** — The characteristics of the symptom-complex following intravenous injection of ammonium salts are well known. In our present experiments ammonium chloride in 10 per cent solution was used throughout, and definite quantities per kilo body weight were injected intravenously. We aimed to make the injection time of the same duration (two minutes) in each experiment, so as to eliminate the variable factors of liver activity, kidney (and other glands) activity, as well as lymph formation.

We found very marked individual variations in susceptibility to the ammonium chloride. Some dogs showed very severe symptoms from doses of 1 or 1.5 c.c. per kilo, others died in tetanic convulsions after injections of 2 c.c. per kilo, while still others recovered from 3 c.c per kilo, the symptoms being only moderately severe.

The hyperexcitability persists for some time after the tremors and convulsions have subsided. The ammonia animal jumps in response to sound or noise very much in the same manner as in strychnine poisoning. This marks a very characteristic difference between the ammonia and the parathyroid tetany, for all our parathyroidectomized animals which have been particularly tested have failed to show hyperexcitability of this type at any stage of the tetany.

2. **Relative action of calcium salts.** — As a means of further comparison, experiments were performed to test the action of calcium salts in ammonia tetany. Our method consisted of injecting varying quantities of calcium lactate corresponding to the average and maximum quantities of calcium used in suppressing parathyroid tetany, and following this immediately or in thirty to sixty minutes by a rapid injection of ammonium chloride (1.5 to 2.5 c.c. per kilo body weight). In each series we made a control experiment, a proportional quantity of ammonia being injected into an animal without previous calcium injection. In two experiments the calcium and the ammonium salts were injected simultaneously. *In no case was the action of calcium in suppressing the ammonia symptoms very marked.* In the series in which 20 c.c. calcium lactate were injected intravenously one half hour before the 1.5 c.c. per kilo body weight 10 per cent solution ammonium chloride, the symptoms appeared slightly less severe and of shorter duration than when no calcium was given. Individual variations in susceptibility to the ammonium salt might explain this apparent slight difference, but could hardly account for the failure of calcium to produce such a marked improvement as it does in the initial stages of parathyroid tetany. Berkeley and Beebe report more positive results. They used a gradual injection of dilute solution of ammonium carbonate, and when the symptoms became severe, the ammonium injection was stopped and a solution of calcium or strontium was injected followed by complete and immediate recovery and a resistance to further injections of ammonia to an extent greater than had been observed previous to the calcium injection. In the case of very gradual injections, the conversion and the elimination of the ammonia are variable factors. This method is further complicated by the depressor phenomena that follow or accompany the ammonia excitation. It would seem that our method is not open to these objections and ought to reveal the antagonistic action, if it were present, of the salts in the

animal. In four experiments we injected calcium during the ammonia tetany, but without apparent change in the severity or duration of the symptoms. Berkeley and Beebe used the carbonate of ammonia in their experiments. The fact that the ammonium chloride is largely eliminated as such by the kidneys while the carbonate is in part changed to urea by the liver might render the calcium salts more efficient in suppressing the symptoms of the latter, by augmenting liver activity.

3. **The effect of transsection of the spinal cord on the parathyroid and the ammonia tetany.** — The work of Horsley,[9] Munk,[10] Lanz,[11] Falta and Rudinger,[12] MacCallum,[13] Biedl and others, seems to indicate that parathyroid tetany is of central origin. Removal of the motor areas of the cerebral cortex diminishes but does not abolish the symptoms. Reflex excitability and direct excitability of the motor nerves are increased. The latter seems to disappear soon after section of the nerves. After cord transsection, increased reflex excitability and spasms posterior to the lesion have been noted in parathyroid tetany. According to Biedl there seems to be no essential difference in the rate of development and the intensity of the symptoms posterior to the cord transsection, whether the transsection is made before the parathyroidectomy or after appearance of the tetany symptoms.

As a control for our ammonia tetany experiments, high thoracic transsection of the spinal cord was made in one dog in parathyroid tetany. The results are best shown by an extract from the protocol.

Experiment No. 11. — Dog ; weight, 8 K.

Jan. 20	Thyroid-parathyroidectomy.
Jan. 21–24 ...	Normal condition, good appetite.
Jan. 25–Feb. 2	Vomiting, depression, tremors, tetany. During this period 13 intravenous injections of calcium lactate (5 per cent) were made in quantities of 20 c.c. to 35 c.c.
Feb. 2, 3.00 P. M.	Strong tremors and tetany. Intravenous injection of 35 c.c. calcium lactate. Most of the symptoms suppressed within five minutes.

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[9] Horsley: British medical journal, 1892, p. 265.

[10] Munk: Sitzungsberichte der Berliner Akademie, 1888 (2), p. 1059.

[11] Lanz: Berliner klinische Wochenschrift, 1898, p. 387.

[12] Falta and Rudinger: cited from Biedl.

[13] MacCallum: Medical news, 1903, lxxxiii, p. 820.

Spinal cord transsected in upper thoracic region under ether anesthesia.

Feb. 3, 7–11 A. M. Slight tremors in shoulder and neck muscles, no tremors posterior to the transsection. Reflex excitability of hind legs good, extensor reflexes particularly easily elicited, anal sphincter slightly patulous.

2.00 P. M. Rapid and irregular respiration, constant tremors of head, neck, and shoulder muscles. Opisthotonus, salivation. No tremors or spontaneous movement behind the transsection.

3.00 P. M. Convulsions and violent tetany of dog anterior to the transsection, accompanied by strong flexion of hind limbs. Animal revived by artificial respiration. Strong pressure on abdomen gives similar flexion of hind limbs. Intravenous injection of 35 c.c. calcium lactate followed by gradual suppression of symptoms in fore part of dog.

10.00 P. M. Return of slight tremors and spasms in fore part of dog.

Feb. 4, 8.00 A. M. Labored respiration, opisthotonus, rigid extension of fore limbs, tremors in face and shoulder muscles. No tremors posterior to lesion, but reflex excitability good. The symptoms increased in severity until 3 P. M.

3.00 P. M. Violent tetany in fore part of dog, artificial respiration given. During the first part of the attack the hind limbs were slightly flexed, and this was followed by rapid extensor spasms. These movements were readily elicited after the recovery from the tetany attack, by strong pressure on the abdomen.

4.00 P. M. 10 c.c. 10 per cent ammonium chloride injected intravenously, followed by extremely severe tetany both in front and behind the transsection. Artificial respiration failed to revive the animal.

It is clearly indicated that in this case the part of the dog posterior to the spinal cord transsection did not take part in the tremors, tonic spasms, and tetany of the fore part of the dog. The movements of the hind limbs, synchronous with the severe tetanic attacks, were not a part of this symptom complex, but were reflexes due to mechanical traction on skin and muscles and to pressure on the abdominal viscera, owing to the intense contraction of the muscles anterior to the transsection.

The absence of the symptoms in the hind part of the dog cannot be ascribed to spinal shock, because the reflex excitability was good, if not actually increased. As stated above, such increased excitability posterior to transverse lesion of the spinal cord has been described by other observers. Taken by themselves, these results would indicate that parathyroidectomy leads, either directly by the absence of a secretion or indirectly by the presence of toxic substances in the blood, to an increased excitability of the entire central nervous system, but that the tremors and tetany symptoms are due to impulses from the brain centres.

We would now invite attention to three similar experiments with ammonia tetany.

Experiment No. 25. — Dog; weight, 7 K.

Feb. 6. Spinal cord transsected in upper thoracic region, ether anesthesia.

Feb. 7, 8.00 A. M. Animal in good condition. Posterior to lesion flexor reflexes easily elicited; extensor reflexes elicited with difficulty. Knee jerk strong, anal sphincter slightly patulous.

2.00 P. M. Condition same as above. 10 c.c. 10 per cent ammonium chloride injected into the saphenous vein in two minutes. Slight transient flexion of the hind limbs and the usual syndrome in fore part of the dog, *i. e.*, rapid respiration, salivation, vomiting, tremors, convulsions, and intermittent tetany, lasting about twenty minutes. Increased reflex excitability, but no spontaneous movements posterior to the transsection.

3.00 P. M. 20 c.c. 10 per cent ammonium chloride injected into saphenous vein, followed immediately by respiratory standstill and extreme tetany in fore part of dog and primary flexion followed by rigid tetanic extension of hind limbs. Artificial respiration failed to revive animal.

Experiment No. 26. — Dog; weight, 6 K.

Feb. 6. High thoracic transsection of spinal cord.

Feb. 7, 8.00 A. M. Reflexes of hind limbs good. Anal sphincter normal.

2.30 P. M. 10 c.c. of 10 per cent ammonium chloride injected into saphenous. Vomiting, rapid respiration, tremors, convulsions and intermittent tetany in fore part of animal; hind part remaining quiescent but showing increased reflex excitability.

2.40 P. M. 10 c.c. of 10 per cent ammonium chloride injected as above with essentially similar results.

2.50 P. M. 25 c.c. of 10 per cent ammonium chloride injected, followed by immediate and fatal tetany both of the fore and the limb part of the animal.

Experiment No. 27. — Dog; weight, 9 K.

Feb. 6. High thoracic transsection of spinal cord.

Feb. 7. 8.00 A. M. Good reflexes posterior to the lesion. Anal sphincter normal.

4.00 P. M. Condition same as above. 25 c.c. 10 per cent ammonium chloride injected intravenously in two minutes. Immediate and fatal tetany of entire animal, but the tetany was less severe in the hind limbs.

The interpretation of the results of these experiments is complicated by the unknown factors of spinal shock. It is possible that the failure of the smaller doses to cause convulsions posterior to the spinal cord transsection is due, at least in part, to a lowered reflex and direct excitability of that part of the cord. But the other alternative seems more probable. While the ammonia increases directly as well as reflexly the excitability of the entire central nervous system, the convulsions and tetany caused by smaller doses of ammonia are probably due to direct or reflex action on higher centres. In the case of moderate ammonia tetany the behavior of the dogs with high spinal transsection is strikingly similar to that of the dog in severe parathyroid tetany and with severed cord. It is to be noted, however, that the parathyroid tetany even of the severest type failed to extend posterior to the cord lesion, while the quantity of ammonium chloride required to produce a tetany of equal severity acts also posterior to the lesion.

III. The Complexity of Parathyroid Tetany.

1. **The symptoms.** — The general character of the syndrome following complete thyroidectomy in dogs has been often described and is well known, but for the purpose of comparison we wish to emphasize the following points.

(1) *Periodicity.* — None of our dogs have died in the first attack of tremors, convulsions and tetany, even when no therapeutic measures were taken. The degree of spontaneous recovery is variable. The dog after a period (one to four hours) of severe tremors, spasms, and respiratory trouble may return to apparently normal condition, eating and playing; usually there is some depression after disappearance of the excitation or symptoms. The duration of this spontaneous recovery varies from a few hours to thirty-six hours. It is usually less than twenty-four hours. The periods of quiescence become gradually shorter as the severity of the symptoms increases. It is obvious that this spontaneous periodicity is a disturbing factor in the study of therapeutic measures that at best have only a temporary action.

(2) *Variability.* — This appears mainly in the relative preponderance or balance of excitatory and depressor symptoms, and of appetite and anorexia. So far we have had only one case (dog) with fatal ending without at any stage showing excitation symptoms. This dog died on the sixth day after the operation. The post mortem revealed lobar pneumonia of the right lung.

(3) *Vomiting-anorexia.* — The vomiting is frequently severe and persistent, even in subjects taking no food. In some cases the vomiting movements pass into convulsions. The anorexia is variable. A dog in tremors will not eat, but may eat heartily an hour later after spontaneous recovery from the excitation symptoms. The excitation symptoms will develop in dogs exhibiting persistent anorexia from the start and therefore taking no food at all after the operation; but the onset seems to be hastened and the severity of the excitation symptoms increased by meat feeding.

(4) *Diarrhea.* — More or less severe diarrhea was present in all of our dogs of this series, — with one exception. The stools are colored brownish-black, with bile, highly offensive and frequently bloody.

(5) *The reflex excitability.* — As is well known, there is increased reflex excitability as well as increased excitability of the motor nerves.

The latter fact is made evident by the stimulation of the phrenic nerves by the action current of the heart, an early observation of Schiff. In most of the dogs showing moderate to severe symptoms the diaphragm, for hours at a stretch, gave a contraction synchronously with the heart. This contraction is obviously a single twitch, while the respiratory contraction is tetanic. The twitch due to the direct stimulation of the phrenics by heart action current cannot be detected during the respiratory contraction, probably because it is merged with this stronger contraction. Its absence may also signify a refractory condition of the nerves when used by the normal discharges.

Despite increased reflex excitability the dogs may show no restlessness. Some of our dogs did show great restlessness, but the usual picture is quiescence and comparative indifference up to and even during the progress of the tremors and spasms. In the later stages of the syndrome the decrease of reflex excitability may be so great that, although able to walk about and responding to call, the animal may pay no attention to severe skin injuries, such as exposing a vein without local anesthesia. Thus, there may exist at the same time *increased motor excitability* as shown by tremors, tetany, and stimulation of the phrenics by the action current of the heart, and *decreased sensory excitability*, as shown by the lack of response to stimuli which under normal condition would be painful.

(6) *Hyperemia, hemorrhages, and ulcers of the stomach and intestinal mucosa.* — The frequency of vomiting, diarrhea, and anorexia directed our attention to the digestive tract. These symptoms may be due to primary disturbances of the gastro-intestinal tract, or to primary changes in the central nervous system. In nineteen out of twenty-three thyroidectomized dogs of the present series we found on post mortem examination marked hyperemia and congestion of the intestinal mucosa, hemorrhagic areas in the mucosa, particularly of the pyloric end of the stomach and of the duodenum, and in a number of cases there were more or less extensive ulcers in the pyloric and duodenal mucosa. In no case did we find that these pathological changes extended to the fundus of the stomach. As regards the intestines, the lesions were always most extensive in the upper part of the duodenum. In a number of cases the pancreas was also greatly congested.

We can, at present, merely record these observed facts without being able to interpret their significance in the parathyroid tetany syndrome. Anorexia is to be expected in an animal with the gastric and intestinal mucosa in the condition usually found in these animals. The anorexia may, however, be primarily of central origin, and the congestion and hemorrhages secondary results of violent contractions in vomiting. The ulcers suggest altered secretions of the digestive glands, a point now under investigation. Fully 90 per cent of the dogs used in our laboratory have intestinal worms, round worms or tape worms, or both. In some of our cases the round worms were massed in the duodenum close to the pyloric end and even in the stomach. The dogs seem to show no obvious digestive disturbances from these parasites under normal conditions.

The above conditions of the gastro-intestinal tract in our parathyroid tetany dogs reminds one of the convulsions and tetany in man associated with acute dilation of the stomach, intestinal infection, and intestinal worms, and other gastro-intestinal disorders. It is not known to what extent this tetany is due to toxic product absorbed into the blood or to irritation of afferent nerve endings, but in late years numerous attempts have been made to bring all types of clinical tetany within the category of parathyroid tetany.[14]

(7) *Circulation.* — The condition of the circulation in our dogs in parathyroid tetany has not been studied in sufficient detail to differentiate all the factors involved. During the spasms and tremors the arterial pressure is usually high, with a rapid and rather feeble pulse. In the extreme tetanic attacks the pulse cannot be palpated.

(8) *Pain.* — Most of our parathyroid tetany dogs show signs of pain (restlessness, groaning) at certain stages of the disease. This seems to be accompanied by excessive tenderness over the thorax and abdomen, especially the latter. Light pressure or stroking of the abdomen increases the groaning, while similar handling of the back, the legs, or the head is without effect. This excessive abdominal tenderness is accompanied by increased tone or contracture of the abdominal muscles.

2. **Measures suppressing the parathyroid tetany symptoms.** — (1) *Calcium and strontium salts.* — The prompt but transitory action of

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[14] HABERFELD: VIRCHOW'S Archiv, 1911, cciii, p. 282; HASKINS and GERSTENBERGER: Journal of experimental medicine, 1911, xiii, p. 314.

the calcium salts in the milder stages of parathyroid tetany has already been discussed. In the few experiments in which intravenous injections of strontium salts were employed the results were practically identical with those obtained with the calcium salts. The main hypotheses advanced by previous workers to account for this action of the calcium salts are (1) The supplying of a deficiency of calcium in the nervous tissue and in the blood; (2) The pharmacologic or depressor action on the nervous system. The second hypothesis may be extended to include depression of afferent nerve endings in case the excitation symptoms are in part reflex.

There is a decrease in the direct excitability of the motor nerves,[15] and the dogs usually exhibit some depression and quiescence for a short period after the calcium injections, but the latter may be a condition of fatigue from the previous hyperactivity rather than due to the direct or indirect depressor action of the salt. If the degree of antitoxic action of the calcium in ammonia tetany is taken as a measure of the extent to which depression of the central nervous system by calcium suppresses convulsions due to a circulating toxine, it is clear to us that the hypothesis does not suffice to account for the action of the calcium and the strontium salts in parathyroid tetany. In five to fifteen minutes after moderately violent tremors and spasms have been suppressed by calcium, the dog may run about, play, eat, and exhibit no obvious signs of depression. This may be accounted for by assuming that in the quantities used the calcium simply brings the excitability of the nervous tissue down to normal, but not below the normal. However, when the tetany symptoms are suppressed by intravenous injections of hypertonic sugar solutions, which in all probability act primarily by depression of the tissues, the dog appears actually depressed even up to the minute the tetany symptoms return.

The suppression of the milder stages of the tetany symptoms by calcium and strontium salts seems too rapid to be explained by an increased elimination or conversion of a circulating toxin; and the possible direct neutralization of the toxin by the calcium must remain a pure speculation until the toxin has been found.

It appears to be true in all cases investigated that the passage of calcium salts into or through cells is relatively slow. The action of calcium

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[15] Quest: Berliner klinische Wochenschrift, 1910, p. 1074. We have confirmed this in two days in parathyroid tetany.

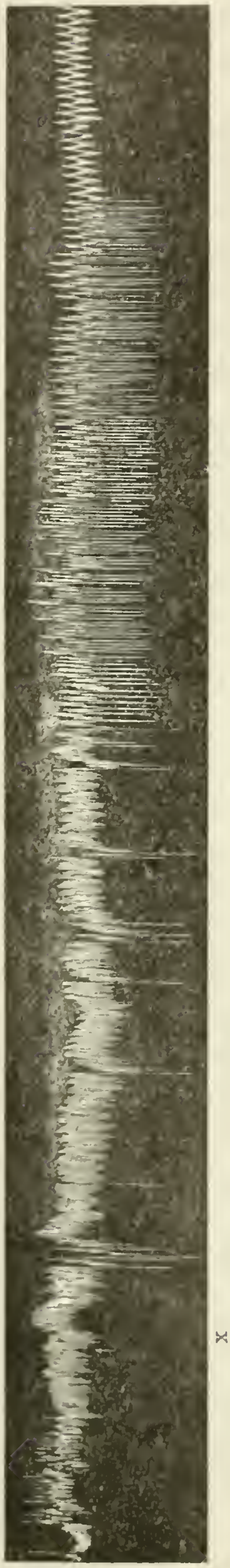

FIGURE 1. — One third the original size. Record of carotid blood pressure in dog A in severe parathyroid tremors and spasm, rapid respiration and salivation. Dog lying down and fairly quiescent. No anesthesia. X, injection of 25 c.c. of 5 per cent calcium lactate into saphenous vein. Showing slowing of the heart and lowering of the blood pressure, *pari passu*, with slowing of the respiration and cessation of the tremors. Carotid pressure before the calcium injection, 170 mm. Hg.

in mild parathyroid tetany is relatively prompt. Can it be a vaso-motor effect, an action on the walls of the arterioles or the capillaries, or on sensory nerve endings in the walls of the blood vessels? The action, in mammals, of intravenous injections of calcium salts upon the heart and the blood pressure is well known. In order to determine whether calcium has the same action, qualitatively and quantitatively, in parathyroid tetany, we isolated one carotid artery at the time of the thyroidectomy so that it could be brought to the surface and a blood pressure cannula inserted without general or local anæsthesia. The edges of the wound were approximated without being stitched, and the neck bandaged until the appearance of the tetany symptoms. So far we have not been able to secure a blood pressure record with the animal in extreme tetany. An illustrative record of the action of calcium on the heart and blood pressure in parathyroid spasms and tremors, not complicated by general or local anesthesia, is given in Fig. 1. The calcium lactate lowers the aortic blood pressure, temporarily, *pari passu*, with slowing of the pulse. The lowering of the general blood pressure is not sufficient to account for the calcium action by diminution of the blood flow through the brain and consequent depression of reflex excitability.

(2) *Organ extracts and tissue proteins.*— In the hands of various investigators the parathyroid tetany symptoms have been temporarily suppressed by hypodermic or intravenous injection of extracts of

thyroid, parathyroid, thymus, pancreas, testes and hypophysis. The thyroid extracts may also contain the active principle or internal secretion of the parathyroid. Beebe[16] has isolated a nucleo-protein from the parathyroids having the same action. Apart from the thyroid and the parathyroid preparations, it is obvious that this alleged action of the tissue extracts in parathyroid tetany cannot be *specific* in the sense of supplying the need of the organism for the parathyroid secretions. Inasmuch as the parathyroid tetany symptoms are suppressed by measures lowering the blood pressure, it would be of great interest to know whether all these organ preparations contain depressor or vaso-dilator substances. If they do, their action may be due solely to this lowering of the blood pressure. We have ourselves tested the action of pituitary extract in two dogs with mild tetany symptoms. Intravenous injections gave in both cases an immediate but slight increase in the symptoms followed by their cessation within ten minutes. In a previous paper we inclined to the view that the alleged action of these various organ extracts in parathyroid tetany is an error of observation due to the spontaneous periodicity in symptoms. It would now seem that the observations may be correct in fact, but that the interpretations of their action have been erroneous.

(3) *Hypertonicity.* — At the recent meeting of the American Physiological Society in New Haven, Joseph and Meltzer reported that intravenous injections of large quantities of hypertonic sodium chloride solution may suppress the parathyroid tetany symptoms in the dog, in some cases after a preliminary excitation. The results of Joseph and Meltzer seem paradoxical, as excitation symptoms are suppressed by a salt which acts itself as a stimulant to the tissues. The quantity and concentration of the sodium chloride solution they used rendered the blood greatly hypertonic and increased its volume, not only by the quantity of fluid introduced, but also by the withdrawal of water from the tissues. According to the reported results the suppression of the symptoms are not as prompt as in the case of calcium injections. If the tetany symptoms are due to circulating toxins, this suppression by the sodium chloride solution may be due to dilution of the toxins or to increased elimination because of the diuresis.

[16] Beebe: This journal, 1907, xix, p. xiii.

We were, however, struck by the fact that the sodium chloride solution had to be so concentrated in order to give the results. A number of years ago, Carlson [17] and Meek [18] showed that hypertonicity, *per se*, depresses ganglion cells, nerve fibres, and skeletal muscle. These facts suggested to us that the results of Joseph and Meltzer may be due to depression, particularly of the nervous tissues, by the hypertonicity. If such is the case, similar results should follow the intravenous injection of hypertonic sugar solutions. This proved to be the case, as illustrated by the following experiment.

Experiment 16. — Dog; weight, 7 K.

Feb. 2, 9.50 A. M.	(Thyroid-parathyroidectomy, Jan. 25.) Strong tremors and spasms. 160 c.c. molecular sugar solution injected into the saphenous vein. There was neither augmentation nor decrease of the symptoms for the first ten minutes, then the symptoms gradually subsided.
11.30	Tremors and spasms completely ceased. Dog seems depressed.
12.00	Tremors begin to reappear with increasing severity.

Hypertonicity by itself may thus temporarily suppress the less severe parathyroid tetany symptoms. That it is a depressor action probably on the nervous tissue, is shown by the state of depression of the animal during the period of quiescence.

(4) *Albumoses* (Witte's Peptone). — Our attention was directed to the possible importance of the circulation in the genesis and the suppression of the calcium and the strontium salts. We had also in mind the older suggestions that certain types of clinical convulsions, epilepsy and tetany may be due to local vaso-motor inco-ordination. It is not probable that the high blood pressure in parathyroid tetany is itself the primary cause of the tetany, and as for primary vaso-constriction, partial anemia, and subsequent hyperexcitability, that is, and probably will remain for a long time, a mere suggestion. The lowering of the blood pressure by substances acting primarily on the walls of the arterioles might suppress the symptoms by relieving such

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[17] Carlson: This journal, 1906, xv, p. 357.

[18] Meek: *Ibid.*, 1907, xvii, p. 8.

constrictor spasms, or by diminution of the blood supply to the brain and spinal cord to a point where decreased excitability sets in.

The following experiments serve to illustrate our results with the peptone injections.

Experiment No. 19. — Dog; weight, 8 K.
Feb. 20. Thyroid-parathyroidectomy.
Feb. 23, 9.00 A. M. Strong tremors, spasms and salivation, rapid respiration.
9.10 A. M. Intravenous injection of 30 c.c. 10 per cent Witte's peptone. Almost immediate diminution in the severity of the symptoms.
9.20 A. M. An additional 20 c.c. of peptone injected.
9.25 A. M. Tremors, spasms, and salivation completely disappeared. Dog greatly depressed and weak. Respiration slow and regular, blood pressure low, heart fairly strong and regular.
2.30 P. M. Dog in good condition, eats and plays; continued in good condition until 10 P. M., when intermittent panting appeared, but no tremors.

In two other experiments the suppression of the tetany by the peptone injections lasted for three and six hours respectively.

It is well established that albumoses or peptones lower the blood pressure. But we are not justified in concluding that this lowering of the blood pressure is the sole or main factor in the suppression of the tetany symptoms. The albumoses act on the liver cells. May they not also act on the nervous tissues in the direction of depression? So far as we know, this question is not yet answered. It is probable that the great vaso-dilation is itself sufficient to depress the brain by partial anemia, and hence suppress the tetany. These results with albumoses in parathyroid tetany render it necessary to control the vaso-motor action of all substances reputed to suppress the parathyroid tetany symptoms. And inasmuch as most, if not all, tissue extracts contain vaso-dilator substances, unless these are removed by special methods, the anti-tetany action of such extracts reported by many observers may be due to their lowering of the blood pressure, in so far as their action is not a mere concomitant of the spontaneous periodicity in the symptom complex. According to Popielski the organ extracts produce anemia of the brain by vaso-dilation, mainly in the viscera.[19]

(5) *Amyl nitrite.* — In pursuance of the study of the circulation in parathyroid tetany amyl nitrite was administered by intravenous injections and in the inspired air.

Experiment No. 18.

Feb. 23, 9.20 A. M. Dog (thyroidectomized two days previously), in strong tremors, salivation, and respiration. Phrenics stimulated by action current of heart.

9.25 A. M. Inhalation of amyl nitrite. Progressive diminution and final cessation of symptoms at 9.30. Inhalation discontinued. Dog seems depressed.

9.40 A. M. Gradual return of the symptoms.

Experiment No. 15. — Dog; weight, 6 K.

Dog thyroidectomized Feb. 13, and used in the interval for tests with strontium and calcium salts.

Feb. 19. 9.30 A. M. Strong tremors and spasms over entire body; dog looks depressed. Pulse, 120, weak, respiration fairly normal.

10.10 A. M. Condition as above; intravenous injection of ¼ c.c. amyl nitrite. Primary increase of spasms, lasting for two minutes, followed by complete cessation of all tremors and quiescence of animal. Dog rather depressed; respiration normal.

10.30 A. M. Dog quite normal, runs about, drinks. Pulse, 140.

11.00 A. M. Strong tremors begin to reappear.

11.10 A. M. Inhalation of amyl nitrite for five minutes, followed by complete cessation of the tetany symptoms.

11.21 A. M. Tremors reappear.

(The inhalation experiment was repeated five times on this dog with results identical to those above.)

We have not tried amyl nitrite in extreme parathyroid tetany. It would in all probability fail there just as calcium and strontium salts fail. The prompt suppression of the milder tetany symptoms by the amyl nitrite inhalation is very striking. The animal becomes at the same time obviously depressed. The obvious explanation of these results is the lowering of the arterial pressure to a point of partial anemia of the brain, and consequent depression of the excitability of the brain and disappearance of the tetany. This vaso-dilator action of the drug is transitory, hence the quick return of the tetany. It is by no means proved that the walls of the blood vessels are the only points of attack of the amyl nitrite. It may depress the brain directly, as well as sensory nerve endings in the blood vessels. If so, this action would in all probability be just as transitory as the vaso-dilator action. These possibilities must be disproved before the above hypothesis can be admitted as proved, or it must be shown by other means that the lowering of the arterial pressure, *per se*, will suppress the tetany.

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[19] Popielski: Archiv für die gesammte Physiologie, 1909, cxxviii, p. 191.

(6) *Section and stimulation of the vagi.* — In this line of attack we aimed at producing simple lowering of the arterial pressure, not attended by any complications, by stimulation of the peripheral end of the cut vagus nerve. Our procedure was as follows. Both vagi were isolated and silk threads passed under them and fixed to the skin at the time of the thyroidectomy, so that the nerves were accessible without anesthesia or pain at any stage of the subsequent tetany symptoms. The following protocols illustrate our results.

Experiment No. 21. — Dog.

Feb. 20. Thyroid-parathyroidectomy.

Feb. 23, 10.00 A. M. Strong tremors; phrenics stimulated by action current of heart; salivation; respiration somewhat accelerated. Right vagus sectioned. *Almost immediate cessation of the tremors and spasms. Heart slowed.* Stimulation of the phrenics by the heart continues. No obvious change in general condition of animal.

10.30 A. M. Tremors begin to return.

11.00 A. M. Strong tremors. Stimulation of peripheral end of cut vagus slows the heart, but the heart "escapes" after less than a minute's tetanizing of the nerve. *The tremors and spasms continue unchanged.* This was repeated eight times with the same results up till 2.00 P. M.

6.00 P. M. Tremors; spasms; salivation; heart stimulation of phrenics have persisted throughout afternoon. Left vagus pulled out through the wound preparatory to section. *During the handling of the nerve the tremors ceased completely;* respiration slow and labored; vomiting, the stimulation of the phrenics by the heart persists. Observed till 7 P. M.; no change in conditions.

9.00 P. M. Tremors, and spasms have returned. Respiration practically normal. Phrenic stimulation present. Left vagus cut. *Immediate and complete cessation of tremors and spasms.*

10.00 P. M. No return of tremors.

12.00 P. M. Stimulation of phrenics by heart not in evidence; but slight tremors present in shoulder muscles.

Feb. 24, 1.00 A. M. Tremors increasing in intensity. Dog killed by ether.

Experiment No. 22. — Dog.

Feb. 27 Thyroid-parathyroidectomized.

Feb. 28, 9.30 A. M. Dog depressed; slight tremors; respiration irregular; symptoms increasing in severity.

11.00 A. M. Strong spasms; salivation. Left vagus cut. *Slight but immediate decrease in severity of the tremors.*

11.10 A. M. Symptoms increasing in severity.

12.00 A. M. Rectal temperature, 42° C.; rapid respiration; salivation; tremors; legs in tonic extension. Right vagus cut, vomiting: *tremors and tonic extension decreased.*

12.15 P. M. Tremors completely ceased; dog runs about; salivation; the rapid respiration interrupted by periods of typical slow respiration.

12.30 P. M. Dog in normal condition, slow respiration.

2.00 P. M. Tetany symptoms returning with rapidly increasing severity.

3.00 P. M. Dog died in extreme tetany while being put under ether preparatory to section of both splanchnic nerves.

Experiment No. 24. — Dog.

Mar. 4.	Thyroid-parathyroidectomy.
Mar. 6, 8.40 A. M.	Tremors; spasms of hind legs; panting; temperature, 40.5° C. Left vagus cut, *slight slowing of respiration. Other symptoms unchanged.*
9.10 A. M.	Right vagus cut. *During the handling of the nerve there was a distinct decrease in the severity of the tremors, and these ceased rapidly after cutting the nerve.* Respiration gradually slowed. Animal runs about; eats.
11.30 A. M.	Animal quiescent; depressed.
12.30 P. M.	Tetany symptoms returning with increasing severity.
2.15 P. M.	Died in tetany while being handled preparatory to injection of calcium; artificial respiration falling.

Experiment No. 23. — Dog.

Mar. 2.	Thyroid-parathyroidectomized.
Mar. 3, 8.30 A. M.	Dog slightly depressed, but eats heartily.
12.00 A. M.	Tremors; irregular respiration.
2.00 P. M.	Strong tremors; panting; salivation; rectal temperature, 40° C. Left vagus cut. *Immediate cessation of tremors in shoulders and forelegs, diminution of tremors in hind legs.* Respiration unchanged. Vomiting.
2.18 P. M.	Tremors returned in entire animal; panting; salivation. Right vagus cut. *Respiration gradually slowed. Tremors gradually subsiding, and interrupted by periods of quiescence, these periods growing longer.*
2.55 P. M.	Respiration practically normal, but the neck muscles show slight tremors; some salivation; eats, but vomits immediately.
3.05 P. M.	Slight transient tremors in hind legs; eats and vomits. No return of tetany up to 10 P.M. when observation ceased. Typical slow respiration.
Mar. 4, 8.30 A. M.	Strong tremors and tetany. Found dead at 2 P. M.

The failure to suppress the symptoms by stimulation of the peripheral end of the vagi, that is, by lowering the arterial pressure through inhibition of the heart, is probably due to the short space of time that the inhibition was maintained. The experiments do not prove that mere lowering of the blood pressure is insufficient to suppress the milder tetany symptoms.

The striking, even if only transitory, suppression of the tetany by the section of one or both vagi, or by mechanical stimulation of the vagi is capable of several interpretations. It may be due (1) to stimulation of the depressor fibres in the vagi: (2) inhibitory impulses (*e. g.*, pain) to the part of the brain primarily concerned in the production of the tetany: or (3) elimination of impulses from the viscera that increase the excitability of the brain. We have no conclusive evidence in support of any of these hypotheses, but in view of the transitory character of the suppression, the stimulation of the depressor fibres appears to us the most probable or essential factor. The rapidity and duration of suppression of the symptoms seem to depend on the severity of the tetany stage. It is probable that in extreme tetany the section or stimulation of the vagi will have no appreciable effect on the symptoms.

The attempts to section both splanchnic nerves in the animals showing strong tetany after section of both vagi, were made with the view of testing the third hypothesis: afferent impulses from the viscera as a factor in the tetany symptoms. But suppression of the tetany by section of both splanchnic nerves would not prove the hypothesis because of the attendant lowering of the blood pressure.

The death in severe tetany at the beginning of ether anesthesia points to the danger of ether and chloroform as a therapeutic measure in severe forms of clinical tetany.

Summary

The ammonia content of the blood of dogs in parathyroid tetany falls within the limits of variations of ammonia percentage in the blood of normal dogs. Intravenous injections of calcium salts in sufficient quantities to completely suppress parathyroid tetany in dogs do not alter the concentration of the ammonia in the blood.

The calcium salts have much less inhibitory action in ammonia tetany than in parathyroid tetany. Dogs in ammonia tetany exhibit a much greater hyperexcitability to auditory stimuli than is the case in parathyroid tetany. On high thoracic transsection of the spinal cord extreme parathyroid tetany (epilepsy) does not directly involve the part of the animal posterior to the lesion, while extreme ammonia tetany involves the whole animal. Ammonia tetany of less severity is confined to the part of the animal anterior to the lesion.

The calcium and the strontium salts appear to be equally efficient in suppressing the parathyroid tetany symptoms. The length of time that the symptoms are kept in abeyance by these salts varies indirectly with the severity of the symptoms and the degree of cachexia. The salts act very rapidly in the case of the milder symptoms, while in the later stages of the disease they have little or no action.

The parathyroid tetany symptoms (except extreme stages) in dogs are suppressed for varying periods by extracts of the hypophysis, hypertonic sugar solution, albumoses, amyl nitrite, section, and stimulation (afferent fibres) of the vagi.

The arterial blood pressure is relatively high in parathyroid tetany. Intravenous injection of calcium lactate shows the heart and lowers the arterial pressure, but not sufficiently to greatly diminish the blood flow through the brain.

Parathyroid tetany in dogs is accompanied by gastro-intestinal disorders: anorexia, vomiting, diarrhea (usually), pain in the abdominal region; and in the majority of our cases hyperemia, hemorrhages, and ulcers of pyloric and duodenal mucosa. The hyper-excitability of the peripheral nerves in dogs in parathyroid tetany is usually, but not always, shown by stimulation of the phrenic nerves by the action current of the heart.

It appears that, with the exception of parathyroid transplantation, all measures that have so far proved efficient in suppressing the parathyroid tetany symptoms are only temporary palliatives. Their action is complicated by the spontaneous periodicity of the symptoms in the early stages of the disease. The efficiency of these measures varies indirectly with the stage of the cachexia, and the severity of the excitation symptoms.

It appears that, with the exception of parathyroid transplantation, the action of all measures that suppress the excitation symptoms can be accounted for by *decreased excitability*, primarily of the nervous tissues. The excitability is decreased *directly* by the drug action of the calcium and the strontium salts and by hypertonicity; indirectly by substances or measures that cause partial anemia of the brain through vaso-dilation (tissue extracts, albumoses, amyl nitrite, stimulation of the depressor nerves). None of these measures have therefore any *specific* significance as regards the cause and nature of parathyroid tetany.

ADDENDUM.

Since our paper went to press two studies on the nitrogenous metabolism in dogs in parathyroid tetany, by Cooke[1] and by Greenwald,[2] have appeared. Both observers found an increase in the undetermined nitrogen in the urine, and Greenwald points out that this indicates liver deficiency. The latter observer finds no appreciable increase in the ammonia content of the blood. This is in accordance with our present results. The data on the urine ammonia are conflicting. The analyses of Cooke show on the whole a great increase, relative and absolute, in the ammonia content, while Greenwald concludes that there is little or no increase in the urine ammonia. The explanations offered by Greenwald to account for these discrepancies should be subjected to experimental verification.

The work of Ott[3] came into our hands also after our paper had gone to press. Ott finds that the parathyroid nucleo-proteid of Beebe greatly lowers the blood pressure. This confirms our surmise that this substance acts through vaso-dilation, just like amyl nitrite, albumoses, and organ extracts in general.

Ott's experiments with extracts of the hypophysis in parathyroid tetany are much more extensive than ours, but with the same general results. Ott's conclusion that this action of hypophysis extract is *specific* is not well founded, in view of our results with depressor substances in general. Neither are we justified in speaking of any of the measures that have so far proved to be temporary palliatives in parathyroid tetany as "*curing*" the tetany.

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[1] Cooke: Journal of experimental medicine, 1911, xiii, p. 339.

[2] Greenwald: This journal, 1911, xxviii, p. 103.

[3] Ott: Internal secretions, Easton, Pa., 1910.

THE VISCOSITY OF BODY FLUIDS AT VARIOUS TEMPERATURES WITHIN PHYSIOLOGICAL LIMITS. — I. THE PROBLEM AND METHOD. — II. VISCOSITY OF BLOOD, PLASMATA AND SERA.

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I.

IN order to understand better the underlying causes of the effects of temperature upon the living organism, it seemed highly desirable by one of us[1] to have, first, a better knowledge about these effects upon the more elementary parts of the organism, and above all upon the physical properties of such parts.

It was with this end in view that the present series of experiments was begun. The first material selected for study was naturally that which is most easily obtained and at the same time that which has the most general importance, namely, blood, and its fluid derivatives. As far as we have been able to ascertain, determinations of the viscosity of these fluids have not yet been made for any considerable range of temperature. The determinations of du Pré Denning and Watson and of Burton-Opitz,[2] for example, extended for the most part only between 30° and 45°. We, on the other hand, have chosen 0° to 45° for our limits, these being generally the bounds of physiological action. Wherever our observations do not reach these limits it will be for the reasons either that the material was no longer in good condition (due to changing temperature effects, or to unaccountable changes from a homogeneous to heterogeneous systems, to speak generally) or for other technical difficulties, which would render further observations no longer comparable.

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[1] SNYDER: This journal, 1909, xxv, p. xxvii.

[2] BURTON-OPITZ: Archiv für die gesammte Physiologie, 1900, lxxxii, p. 470; DU PRÉ DENNING and WATSON: Proceedings of the Royal Society, Series B., 1906, lxxviii, p. 328.

II.

Since the object of these experiments is to know the *relative* influence of temperature, only *relative* viscosities have been determined. The method employed, therefore, has been essentially that of Ostwald.[3] The viscosity tubes used were made after the pattern of that author, as well as the thermostat and thermo-regulator, modified according to Beck and Hirsch.[4] The viscosimeter and pressure apparatus of these latter authors we have used also, but to no particular advantage. The tubes devised by du Pré Denning and Watson (*loc. cit.*) to prevent the settling of blood corpuscles have not yet been used, since we have not yet worked with whole blood extensively.

The method requires that the specific gravity of the fluids examined be known. For the present investigation it was held to be essential that the specific gravities be determined as accurately as possible and at several different temperatures. The only satisfactory method for this purpose is that recommended by Ostwald,[5] where the Sprengel-Ostwald pyknometer is employed. The simpler methods of Hammerschlag[6] and of Jones[7] were only used as a check, or wherever there seemed to be no value in a determination closer than the second decimal place.

To make a single specific gravity determination of a specimen of blood or blood plasma at room temperature with a Sprengel-Ostwald pyknometer is a task simple enough. But to make a series of determinations of such a specimen at intervals of 5 degrees from 0° to 45° and then to take viscosity readings for the same specimen through the same thermal gamut, — this we found to be a most difficult task. And while we have been able to complete almost the series in one or two cases, we have no experiments as yet to report where there are no gaps at all.

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[3] Ostwald-Luther: Physiko-chemische Messungen, Leipzig, 3te Aufl., 1910, p. 231.

[4] Beck und Hirsch: Münchener medizinische Wochenschrift, 1900, Nr. 49.

[5] Ostwald-Luther: *Loc. cit.*, p. 177.

[6] Hammerschlag: Zeitschrift für klinische Medezin, 1892, xx, p. 444.

[7] Jones: Journal of physiology, 1891, xxii, p. 299.

The capacity of the pyknometer must first be accurately determined for each temperature at which a specific gravity determination is to be made. The various weights of distilled water which this vessel holds at each of the given temperatures, together with coefficients for correcting the differences in weight of the empty tube at room temperature and at the new temperature, for air held in solution by the water, etc.,[8] these are the data from which one calculates the volume of the tube at the given temperature. The determination of the specific gravities of the fluid under examination becomes now a mere matter of routine. The specific gravities of water at various temperatures, as determined by Thiessen, Scheel and Diesselhorst[9] (1900), are made use of as shown below.

The relative viscosity of the fluid is then calculated from the well-known formula,

$$\eta_1 = \eta_0 \cdot \frac{s_1\, t_1}{s_0\, t_0},$$

where s_1 and t_1 represent the specific gravity and the time of flow, respectively, of the fluid examined; s_0 and t_0, the same quantities for distilled water; and η_0 represents the absolute viscosity of water. Of course, all the values must be taken for some given temperature and pressure.

For values of η_0 we took the temperature determinations of Thorpe and Rodgers.[10] To put η_0 equal to unity, as is sometimes done, or to the viscosity coefficient of water at 37° in every case, as was done by du Pré Denning and Watson, would give results worthless for comparison in this work.

Having determined the viscosity of a given fluid at each temperature according to the method just described, a *temperature coefficient of the viscosity* may then be calculated.

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[8] Börnstein: In Landolt-Börnstein-Meyerhoffer, Physikalisch-chemische Tabellen, 3te Aufl., 1905, p. 42.

[9] Thiessen, Scheel und Diesselhorst: *Ibid.* and Wissenschaftliche Abhandlungen der Physik. — Technische Reichsanstalt, 1900, iii, p. 68.

[10] Thorpe and Rodgers: Philosophical transactions, 1894, A, clxxxv, p. 397; Proceedings of the Royal Society, 1894, lv, p. 148.

III.

In the following table are brought together the results of our determinations of dog peptone plasma.[11] The table contains the data necessary for the calculation of (1) the viscosity at any temperature; (2) the temperature coefficient for intervals of 10 degrees.

TABLE I.

THE VISCOSITY OF DOG'S PEPTONE PLASMA AS INFLUENCED BY TEMPERATURE (VISCOSIMETER NO. IV).

DISTILLED WATER.				DOG'S PEPTONE PLASMA, SPECIMEN NO. 2.				
Temperature. C.	Absolute viscosity. Thorpe and Rodgers (η_0)	Time of flow in seconds. Tube iv (t_0)	Time of flow times specific gravity ($s_0 t_0$)	Specific gravity (s_1)	Time of flow in seconds. Tube iv (t_1)	Time of flow times specific gravity ($s_1 t_1$)	Relative viscosity (η_1) $\eta_1 = \eta_0 . \frac{s_1 t_1}{s_0 t_0}$	Temp. coef. for intervals of 10° (Q_{10})
0	.01778	280	280	1.0246	573	587	.03726	....
5	.0151	237	237	....	493	504	.03201	....
10	.0130	205	205	1.0235	411	420	.0265	1.48
15	.0113	180	180	....	348	355	.02254	1.42
20	.0100	158	158	1.0179	306	311.5	.01973	1.34
25	.0089	140	139.6	....	266	270.6	.01719	1.31
30	.0080	126	125.5	1.0172	240	244	.0155	1.27
35	.0072	114	113	....	213	216	.01376	1.25
40	.0065	104	103	....	190	193	.01242	1.24
45	.00597	....	....	....	....	....	....	....

This table contains all the data necessary for the calculation of the values found in the last two columns, excepting the specific gravity of water, and will suffice as an illustration of the derivation of the results of the following determinations.

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[11] The authors have to thank Mr. H. L. CECIL, working with Professor HOWELL, for the very efficient peptone used for this plasma.

Of dog's oxalate plasma (sp. gr. at 15°, 1.031):

temp. =	0°		10°		20°		30°
η_1 =	.0346		.0240		.0181		.0141
Q_{10} =		1.42		1.32		1.28	

Of cat's serum:

temp. =	0°		10°		20°		30°
η_1 =	.0294		.0209		.0156		.0121
Q_{10} =		1.40		1.34		1.29	

Of hog's serum:

temp. =	20°	25°	30°	35°	40°	45°
sp. gr. =	1.0215	....	1.0184	....	1.0152	....
η_1 =	.0188	.0163	.0143	.0125	.0111	.0097
Q_{10} =	....	1.31	1.30	1.29	1.288	....

Of hog's defibrinated blood:

temp. =	20°	25°	30°	35°	40°	45°
sp. gr. =	1.0598	....	1.0566	....	....	1.0518
η_1 =	.095	.0729	.0572	.046	.0382	.035
Q_{10} =	....	1.66	1.58	1.37	1.31	....

Using the Beck and Hirsch pressure apparatus attached to the viscosimeter, the following results were obtained for a specimen of peptone blood of the dog. Of course in this case the time of flow of the blood had to be compared with the time of flow of some fluid more viscous than water. Aniline oil was chosen, the specific gravity and viscosities of which, at intervals of 5 degrees from 0° to 45°, had to be determined beforehand. The latter determinations being more purely physical-chemical studies will be published elsewhere. The results by this method are as follows:

Of (whole) blood of the dog, peptonized:

temp. =	0°		10°		20°		30°
η_1 =	.1173		.0696		.044		.0338
Q_{10} =		1.68		1.58		1.30	

SUMMARY.

The results of this study may be summarized at this point as follows:

1. Like the viscosity of water and other fluids of definite chemical composition, the viscosity of blood, plasmata and sera, increases with fall of temperature. This corroborates and extends the findings of Burton-Opitz and of du Pré Denning and Watson.

2. The temperature coefficients of the viscosity of blood, plasmata and sera, for intervals of 10 degrees varies with the temperature, the larger coefficients being for the lower and the smaller for the higher ranges of temperature.

3. This variation of temperature coefficient is in the same direction and of the same character as the variation of the temperature coefficient of velocities of chemical reactions and of physiological actions. A comparison and discussion of these facts will be found in another paper.

ON THE MEANING OF VARIATION IN THE MAGNITUDE OF TEMPERATURE COEFFICIENTS OF PHYSIOLOGICAL PROCESSES.

By CHARLES D. SNYDER.

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I.

IN a former note[1] it was briefly pointed out that one of the characteristics of the temperature coefficients of physiological processes is the tendency for their values always to grow greater for the lower and smaller for the higher ranges of temperature. This is brought out most strikingly in Table I, where the coefficients for very different processes are shown.

Many more examples could be added to the list. In fact, the writer knows of no case where this variation does not occur. Now in cases where the mean value of the coefficients lies between 2 or 3, it has been argued that, on account of this variation, such coefficients cannot be regarded as evidence of underlying chemical action. It is for this reason, for example, that Mlle. Filon,[2] working under the direction of M. L. Lapique, concludes that "one may not make use of the influence of temperature upon excitability as proof that the essential process of excitation is a chemical phenomenon." The values of Q_{10} in this author's experiments on excitability of frogs' sartorius were 4.2 between 5° and 15° and 2.0 between 15° and 25°; of frogs' gastrocnemius, the values were 2.0 between 5° and 15° and 1.4 between 15° and 25°.

However correct the logic in this argument may be, the conclusion is invalid because the major premise upon which it is based is, as a matter of fact, incorrect. That is, the value of Q_{10} for purely chemical reactions is not always constant, as the author quoted supposes, but varies in many cases as it does for physiological actions, the value of Q_{10} being greater for the lower ranges than for the upper ranges of temperature. To see this, one needs only refer to the literature.

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[1] SNYDER, C. D.: Proceedings of the American Physiological Society, 1909. See This journal, 1909, xxv, p. xxvii.

[2] FILON, GENEVIÈVE: Journal de physiologie et de pathologie générale, 1911, xiii, p. 19.

TABLE I.

Temperature coefficient for	k at 10° / k at 0°	k at 15° / k at 5°	k at 20° / k at 10°	k at 25° / k at 15°	k at 30° / k at 20°	k at 35° / k at 25°
Rate of beat of isolated heart of the turtle . . .	10.2	3.5	2.2	2.1	1.9	1.4
Rate of beat of the isolated sinus venosus of the frog	(3.5)	2.9	2.6	2.1	1.6	(1.5)
Velocity of nerve conduction, — ischiadicus of the frog	(2.7)	2.7	2.3	1.8	1.8	1.5
Respiratory rate in larvæ of dragon flies on low oxygen supply (Babák & Rocek)[3]	...	2.7	2.0	1.9	1.8	1.5
Coagulation time of blood (Addis)[4]	5.0	4.0	3.8	2.7	2.3	2.5
Plasmolysis of plant stems (van Rysselberghe)[5] . .	3.5	3.3	1.8	1.2	1.1	...
"Muscle rhythm" in turtle, normal innervation (Piper)[6]	...	2.0	1.9	1.6	1.4	1.4

But since this point is one upon which the burden of this paper chiefly rests, the writer will take the liberty of reviewing the evidence in some detail.

The values of Q_{10} in the reaction of monochloracetic acid and glycolic acid,[7] and of the reaction $2\ \mathrm{Fe}'' + 2\mathrm{H} + \mathrm{ClO}'_3 = 2\ \mathrm{Fe}''' + \mathrm{ClO}'_2 + \mathrm{H_2O}$ as observed by Hood [8] and by Noyes and Wason,[9] are greater for the lower and smaller for the higher temperatures. This is true also for the reaction

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[3] BABÁK und ROCEK: Archiv für die gesammte Physiologie, 1909, cxxx, p. 477. See Table III.

[4] ADDIS: Quarterly journal of experimental physiology, 1908, i, p. 305.

[5] VAN RYSSELBERGHE: Bulletin de l'Académie royale de Belgique, 1901, xxxix, p. 173.

[6] PIPER: Archiv für Physiologie, 1910, p. 207.

[7] SCHWAB: Recueil de travaux chimique Pays-Bas, 1883, ii, p. 164. Quoted from TRAUTZ and VOLKMANN.

[8] HOOD: Philosophical magazine, 1885, xx, p. 185.

[9] NOYES AND WASON: Zeitschrift für physikalische Chemie, 1897, xxii, p. 210.

$$H_2O_2 + 2\ HI = I_2 + 2\ H_2O,$$

observed by Harcourt and Esson.[10] The values for this reaction are:

t =	0°		10°		20°		30°		40°		50°
k =	1.00		2.08		4.32		8.38		16.19		30.95
Q_{10} =		2.08		2.07		1.94		1.93		1.91	

In the hydrolysis of nitrobenzamide, as reported by Remsen and Reid,[11] we have:

t =	60°		70°		80°		90°		100°
k =	0.058		0.107		0.184		0.305		0.485
Q_{10} =		1.84		1.72		1.65		1.59	

Bugarzsky's[12] velocities in the reaction of bromine upon ethyl alcohol give these values:

t =	0°		10°		20°		30°
k =	.0069		.0210		.0640		0.182
Q_{10} =		3.05		3.04		2.85	

For the reaction of methyliodide and sodiumethylate as observed by Hecht and Conrad,[13] and arranging the coefficients seriatim from lower to higher temperature, we have the following:

3.48 3.40 3.26 3.22 3.36

For the intramolecular reactions[14] (1) in anissynaldoxim, we have 3.41, 2.58, 2.25; (2) in anisaldoximacetate, 2.42, 2.30, 2.07.

In the transition of diazoamido into amidoazo compounds, we have the following:[15]

t =	25°		35°		45°		55°
k =	.006		.0246		.081		.253
Q_{10} =		4.1		3.29		3.12	

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[10] Harcourt and Esson: Philosophical transactions, 1867, clxvii, p. 117.

[11] Remsen and Reid: American chemical journal, 1899, xxi, p. 281.

[12] Bugarzky: Zeitschrift für physikalische Chemie, 1903, xlii, p. 545.

[13] Hecht und Conrad: *Ibid.*, 1889, iii, p. 450.

[14] H. Ley: *Ibid.*, 1895, xviii, p. 376.

[15] Goldschmidt und Reinders: Berichte der deutschen chemischen Gesellschaft, 1899, xxix, p. 1369.

For the case of saponification of ethyl acetate as shown by Warder[16] the values of Q_{10} are:

2.21 1.97 1.87 1.76;

and those for the hydrolysis of carbonsulphid from the work of Buchböck[17] are:

3.87 3.5 3.6 3.35

In view of the very small coefficients obtained by Kooij[18] in the decomposition of arsine and phosphine, namely, 1.2, between 250° and 500°, van't Hoff[19] thought that the value of Q_{10} would be found to diminish gradually if observations could be made over sufficiently great ranges of temperature. That the decrease may still go on is shown by the results of Bodenstein[20] for the decomposition of hydrogen iodide:

t =	300°–310°	400°–410°	500°–510°
Q_{10} =	1.89	1.64	1.53

Using these facts as a starting point, Trautz and Volkmann[21] made a special investigation of the phenomenon. They used the reaction, saponification of fatty acid esters in dilute solutions, as material. In all cases they found a general tendency for the value of Q_{10} to diminish with rise of temperature.[22]

The magnitude of the temperature coefficient for differences of 10 degrees varies, then, not only for physiological actions but also for many chemical reactions. In both orders of phenomena the variation is in the same direction.

II.

This similarity of variation in both cases is remarkable, and one asks at once as to what the causes thereof may be.

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[16] WARDER: Berichte der deutschen chemischen Gesellschaft, 1881, xiv, p. 1365.

[17] BUCHBÖCK: Zeitschrift für physikalische Chemie, 1897, xxiii, p. 156.

[18] KOOIJ: *Ibid.*, 1893, xii, p. 161.

[19] VAN'T HOFF: Studien zur chemischen Dynamik, 1896, p. 138.

[20] BODENSTEIN: Zeitschrift für physikalische Chemie, 1899, xxix, p. 314; 1894, xiii, p. 56.

[21] TRAUTZ und VOLKMANN: *Ibid.*, 1908, lxiv, p. 53.

[22] The maximum value of Q_{10} which they found at 10° seems to be a peculiarity of the concentration of the reagents, namely, 1/100 normal. No such distinct maximum is obtained in concentration of 1/10 or greater.

In the case of chemical reactions van't Hoff[23] suggested that the variation may be due to changes in the viscosity of the solvent, and Buchböck[24] was able to show that if the viscosity of the solvent were increased by the addition of indifferent substances (temperature remaining constant) the velocity of the reaction could be perceptibly slowed. Trautz and Volkmann[25] showed that a very satisfactory mathematical formula for the influence of temperature upon saponification of fatty acid esters could be developed by introducing, among others, a differential factor for the viscosity of the water used as a solvent.

On the other hand, consideration of the influence viscosity may have upon the character and velocity of physiological action has not been entirely neglected. Durig[26] showed that a diminution in the velocity of nerve conduction took place in frogs whose tissues had a smaller percentage of water and, presumably, a higher viscosity. Demoor and Philippson[27] could simulate the effects of temperature upon the action of frogs' muscles by merely changing the tonicity of the baths in which they were kept. They attributed their results to the differences of viscosity and surface tension produced when the baths abstracted water from, or gave water to, the tissues.

In a most interesting paper on a physical theory of nerve conduction, and one which has largely stimulated the author to write the present paper, Sutherland[28] has shown that the velocity of the impulse at any temperature, within physiological limits, may be simply a function of the viscosity of water at that temperature. That is, the variation in conduction may be due only to a variation in the viscosity of the chief (?) solvent of the nerve substance.

On account of the lack of suitable method, actual experimental data obtainable concerning the viscosity of living tissues have been confined to those parts that can be studied in a fluid state. The literature and results of the chief authors are briefly referred to in an article which appears in the present number of this journal.[29]

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[23] VAN'T HOFF: Studien zur chemischen Dynamik, 1896, p. 138; Vorlesungen über theoretische Chemie, 1901, i, pp. 215 ff.

[24] BUCHBÖCK: *Loc. cit.*

[25] TRAUTZ und VOLKMANN: *Loc. cit.*

[26] DURIG: Archiv für die gesammte Physiologie, 1902, xcii, p. 293.

[27] DEMOOR et PHILIPPSON: Archives internationales de physiologie, 1908, vi, p. 210.

[28] SUTHERLAND: This journal, 1908, xxiii, p. 115.

From these results it can be found that if one takes quotients for the viscosity constants observed at intervals of 10 degrees, one gets a series of coefficients which, like the values of Q_{10}, of chemical and physiological actions, are larger for the lower and smaller for the higher ranges of temperature. What is most remarkable for the present purpose is the fact that the coefficients of blood plasma are of nearly the same magnitudes and vary to the same extent and in the same direction as do the viscosity temperature coefficients for water and egg albumin. This is shown in the following table.

TABLE II.

QUOTIENTS OF VISCOSITY COEFFICIENTS TAKEN AT INTERVALS APART OF 10 DEGREES TEMPERATURE

Substance	0/10	5/15	10/20	15/25	20/30	25/35	30/40
Water (after Thorpe and Rogers)	1.36	1.34	1.30	1.27	1.26	1.24	1.22
Egg albumin (after A. J. Ewart, quoting W. Sutherland . .	...	1.29	1.21	...	1.20	...	1.14
Peptone plasma of dog's blood (Snyder and Todd)	1.48	1.42	1.34	1.31	1.27	1.25	1.24

It can be safely assumed that the fluid substances of muscle and nerve, for example, have a viscosity coefficient of the same order, and are most probably affected by temperature in the same way, as blood plasma. Does this account entirely for the great variations in the values of Q_{10} which one finds for the influence of temperature upon physiological actions?

III.

In order to find, if possible, a case where the values of Q_{10} varied less, experiments were carried out on the influence of temperature upon the latent period and shortening phase of strips of turtle's ventricle. The experiments were done in the fall of 1909.[30]

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[29] SNYDER and TODD: This journal, 1911, xxviii, p. 161.

[30] The results of these experiments were merely hinted at in a former note. See SNYDER: *Loc. cit.*

Strips of ventricle were chosen for material (1) because of its comparative simplicity, (2) because no complete data of the sort desired could be found in the literature on ventricle strips.

The strips were suspended in moist-air chambers from writing levers. In this condition they do not beat spontaneously, as is well known.

The technique employed was that used for recording latent period and contraction curve of simple frog-muscle preparation, stimulating with maximal break induction shocks. The method of regulating the temperature was the same as that used in the author's work on turtle's isolated heart and frog's sinus. The results are tabulated below, being typical observations in nine different series of experiments. The individual variation of the velocities was in no case considerable. Here again one sees the wide divergences of the values of Q_{10} as one passes from 0 degrees upward.

TABLE III.

THE TIME OF LATENCY AND SHORTENING OF TURTLE'S VENTRICLE STRIPS AT VARIOUS TEMPERATURES AND THEIR TEMPERATURE COEFFICIENTS.

Temperature	0°	5°	10°	15°	20°	25°	30°	35°
Latency of contraction in seconds	2.0	0.96	0.40	0.24	0.14	0.09	0.06	0.07
Temperature coefficient of latency	..	5.0	4.0	2.85	2.66	2.33	1.28	..
Shortening phase in seconds	14.0	6.0	4.0	2.4	1.6	1.1	0.8	..
Temperature coefficient of shortening phase . . .	..	3.5	2.5	2.5	2.18	2.0	..	..

IV.

At this stage, and as a concluding chapter, it is pertinent to compare concisely the limits of variation of the three orders of phenomena, taking a type from each order and coefficients within the limits of temperature consistent with physiological action.

(1) The values of Q_{10} for the viscosity of water vary from 1.36 to 1.22.
(2) The values of Q_{10} for reaction between $H_2O_2 + 2HI$ vary from 2.08 to 1.91.
(3) The values of Q_{10} for the latency of contraction of ventricle strip vary from 5.0 to 1.28.

Of these phenomena, that of viscosity is simplest in its nature, and we may regard the coefficients observed for it as representing purely viscosity relations of the substance considered. Not so with chemical reaction, for besides the intra- or extramolecular exchange, the velocity of the phenomenon depends, as we have seen, also upon viscosity. Now, since viscosity is the simpler process in the action, and since the coefficients of viscosity are variable, we may infer that the variation of coefficients in chemical reaction is due to the variation in viscosity, the real temperature coefficient of the chemical reaction being itself a constant. This constant may be determined empirically from the *observed* coefficients of the chemical action and the known coefficients of the solvent. For the reaction, $H_2O_2 + 2HI$, this constant is about 1.56. The *observed* and calculated values of Q_{10} then of the reaction are, for temperatures between 0° and 50°, as follows:

Q_{10} observed =	2.08	2.07	1.94	1.93	1.91
Q_{10} calculated =	2.12	2.03	1.96	1.90	1.86

Here the calculated value of Q_{10} in each case is the product of the constant, 1.56, into the viscosity coefficient at corresponding temperature. See Table II (water). The calculated values agree pretty well with the observed values.

Without claiming that the above treatment has any significance in reality, one may, at any rate, say that the ultimate explanation of chemical reaction velocities will be a comparatively simple one.

Remembering at the same time that Professor William Ostwald[31] has referred to the influence of temperature upon chemical reactions as "one of the darkest chapters in chemical mechanics," what shall we say of the influence of temperature upon physiological actions? Although, since Ostwald's opinion was uttered, something has been said also about the influence of temperature upon physiological action, the present author believes that the matter is more complex than even that of chemical reaction, dark as that may be, and cannot believe with Sutherland[32] that viscosity is the only variable factor with the temperature (for velocity of nerve conduction.)

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[31] Quoted after MELLOR, Chemical statics and dynamics, 1904, p. 383.

[32] SUTHERLAND: *Loc. cit.* One important objection to Sutherland's theory of nerve is this: if the nerve impulse is a "propagation of shear in the nerve substance," dead nerve ought to show a demarcation and action current, as well as live nerve. This, however, as is well known, is not the case.

After a careful review of the facts at hand it seems preferable to adhere to the hypothesis put forth in a former paper,[33] namely, that even in certain of the simpler physiological actions we still have to deal with at least two distinct chemical actions whose fundamental velocities at any given temperature are different. A common temperature coefficient would not alter this difference in velocities.

The physiological action, being a resultant of the chemical reactions, might then have temperature coefficients which would vary as those found by experiment.

For further details in regard to this explanation the reader is referred to the original communication.

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[33] SNYDER, C. D.: This journal, 1908, xxii, pp. 195–201.

CONTRIBUTIONS TO THE PHYSIOLOGY OF LYMPH.— XVI. ON THE LOCAL HEMODYNAMIC ACTION OF TISSUE METABOLITES.

By A. J. CARLSON, A. WOELFEL, and H. W. POWELL.

[*From the Hull Physiological Laboratory of the University of Chicago.*]

IN the previous publications of this series new evidence has been produced that seems to render the factors of filtration, transudation, and osmosis, as these processes are understood to-day, incapable of accounting for the production of lymph.[1] Lymph contains an excess of chlorides as compared with the serum of the same animal. The lymph is being continually poured into the blood. The excess of chlorides in the lymph cannot come from the tissue cells, because in that case the cells would be depleted of chlorides. The excess of chlorides must therefore come directly from the blood serum. Whatever be the mechanism effecting this chloride concentration in the lymph,[2] the fact itself renders the filtration theory of lymph formation untenable. The tissue cells (and this includes the endothelium of the blood capillaries) must therefore be looked upon as the primary or essential factors. The tissue cells may do this work by means of the osmotic pressure of the metabolites either within the cells or after being discharged into the tissue lymph (Starling, Asher); or some of the metabolites may act as hormones or secretagogues to the blood and the lymph capillaries (Carlson). That the mechanism is not osmosis seems to be shown by the fact that the lymph continues to be produced under conditions where the osmotic pressure of the serum is much greater than that of the lymph, and by the further fact that the greater osmotic pressure of normal lymph as compared to serum is due to the excess of chlorides rather than to tissue metabolites. We have already pointed out that these chlorides come from the serum and not from the tissue cells. The second hypothesis led to the discovery that lymph itself has a lymphagogue action not dependent on blood pressure changes. This is interpreted as due to the stimulating action of tissue lymphagogues on the cells of the blood and lymph capillaries.

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[1] Carlson, Greer, and Becht: This journal, 1907, xix, p. 360; 1908, xxii, p. 104; Carlson, Greer, and Luckhardt: *Ibid.*, 1908, xxii, p. 91; Luckhardt: *Ibid.*, 1910, xxv, p. 345.

[2] Hamburger: Zeitschrift für Biologie, 1893, xxx, p. 143; Osmotischer Druck und Ionenlehre, 1904, ii, p. 54; Starling, Journal of physiology, 1894, xvi, p. 224.

The processes of formation of tissue lymph are to a great extent independent of the processes of formation of lymphatic lymph, at least in some of the organs of the body. Thus, in the active salivary glands the rate of formation of tissue lymph from the blood must be nearly as great as the secretion of the saliva, but these processes do not necessarily involve increased production of lymphatic lymph, as judged by the output of lymph from the lymphatic ducts of the glands.

The correlation of blood supply with tissue activity involves four factors: (1) oxygen supply; (2) water supply; (3) material for oxidation and tissue repairs; (4) waste removal. In the case of organs whose activity yields a watery external secretion (salivary, lachrymal, and mammary glands, stomach and intestinal mucosa, kidney, lungs, liver), the circulatory correlation in activity with reference to the water supply is probably of greater importance than in the case of the organs having no other external secretion than the lymphatic lymph. The rapid withdrawal of water from the blood in the active salivary glands would probably lead to a concentration of the blood to a point involving impairment of the activity of the organs, unless the quantity of the blood flowing through the organ is greatly increased. It is well known that the increased flow through the active salivary glands is so great that the venous blood is actually less venous than when the gland is quiescent, despite the greatly increased oxygen consumption and carbon-dioxide production. This seems like a correlation with reference to the water supply rather than to the oxygen supply and the waste removal. In fact, when the blood supply to the active salivary glands is diminished, it is the secretion of the water rather than the solids of the saliva that is retarded.[3]

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[3] Carlson, Greer, and Becht: This journal, 1907, xx, p. 180.

It is now generally admitted as proven that reflex nervous mechanisms related to the musculature of the arterioles are factors in the correlation of organ activity with blood supply in the cases of most if not all of the organs in the vertebrates. But it has not been proved that the heart nerves and the vaso-motor nerves are the only factors. This was clearly recognized by Gaskell thirty years ago. "It is surely worth the while," he says, "to see whether it is not possible that the chemical changes going on in the organ itself may not directly bring about dilation of the blood vessels of that organ, and so, without the intervention of the nervous system, regulate its own blood supply according to its own needs." [4] Gaskell showed that weak acids diminish the tone of the blood vessel musculature, while weak alkalies increase the tone. He then postulates the hypothesis that the muscle fibres of the arterioles, bathed directly in the lymph of the organ, are acted on directly by the tissue metabolites in the lymph rather than indirectly after having entered the blood stream. It was held that organ activity involved the production of acid bodies. According to Gaskell these acid bodies diminish the tone of the musculature of the arterioles and thus bring about a local increased blood flow. Gaskell considers briefly the possibility of the action being confined to the capillary walls, but rejects this view as insufficient or untenable.

Bayliss and Starling have reported that intravenous injection of boiled extracts of organs cause vaso-dilation of corresponding organs, and they put forth the hypothesis that the normal vaso-dilation accompanying organ activity is, in part, due to specific sensibility of the arterial walls to the metabolites of the organ which they supply.[5] Thus injection of muscle extract causes vaso-dilation mainly in the limbs, etc. According to this view, the metabolites enter the blood in the capillaries, pass on to the general circulation, and on being returned to the organ whence they came, act on the walls of the arterioles in a way to diminish the tone of the musculature. This involves an organ specificity of the muscular or neuro-muscular apparatus of the arterioles. For if the metabolites act on the arterioles in general, their action would defeat their own end: the increased blood supply to the specific organ in the state of increased activity. The hypothetical "vaso-dilatin" (tissue extracts) of Popielski is also considered to act on the arterial walls.[6] Vincent and Sheen[7] were unable to obtain the vaso-dilation from organ extracts reported by Bayliss and Starling. The observations of May[8] on the blood flow in the active but denervated pancreas seem to show vaso-dilation as a normal sequence of activity. According to May, the tissue metabolites act on the musculature of the walls of the arterioles as general vaso-dilators. The reason given for their acting on the pancreas alone in the case of pancreas activity is that "they are formed locally, and are present therefore in the pancreas alone in a form sufficiently concentrated to produce their effect." It is not clear whether May adopts the hypothesis of Gaskell or that of Bayliss and Starling. Henderson and Loewi[9] interpret the vaso-dilator action that accompanies the salivary secretion produced by pilocarpin as due to metabolites from the gland activity, and so far as we can make out from the footnote in which the question is discussed, they follow the view of Bayliss and Starling as to the point of action of these vaso-dilator metabolites. At any rate, they do not specifically point out that the metabolites may increase the blood flow by direct action on the capillaries, although in a previous paper they argue that the increased blood flow through an atropinized submaxillary gland, inclosed in a plaster of Paris cast, produced by chorda stimulation may be caused by the action of dilator nerve fibres on the blood capillaries. The results of their experiments require no such hypothesis. When the gland is incased in a plaster of Paris cast we have essentially the same circulatory conditions as in the brain with the skull intact. Increased blood flow through vaso-dilation, be it arterioles or capillaries, can be produced only by the compression of the veins or the expulsion of the lymph (or cerebro-spinal fluid), and secretion. The existence of vaso-dilator nerve fibres to the walls of the arterioles certainly seems more firmly established than to the capillaries, and arterial dilation alone will account for all the facts.

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[4] Gaskell: Journal of physiology, 1880–1882, iii, p. 48.

[5] Bayliss and Starling: *Ibid.*, 1902, xxvii, p. 351.

If local vaso-dilation due to the tissue metabolites or specific hormones acting on the capillary walls or on the arterioles is a factor of importance in organ activity, it would seem (1) that organs demanding the greatest increased blood flow in activity should contain or produce more of these hormones than organs requiring less augmented blood flow, and (2) that the concentration and production of these hormones in the organs should diminish with activity. We have tested the hypothesis along these two lines in the following way.

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[6] Popielski: Archiv für die gesammte Physiologie, 1908, cxxvii, p. 191.

[7] Vincent and Sheen: Journal of physiology, 1903, xxix, p. 242.

[8] May: *Ibid.*, 1904, xxx, p. 408.

[9] Henderson and Loewi: Archiv für experimentelle Pathologie und Pharmacologie, 1905, lii, p. 62.

PREPARATION OF THE ORGAN EXTRACT.

Equal quantities of the organs from the same dog were ground up in washed sand, extracted for ten minutes with equal quantities of Ringer's solution, filtered through cheese cloth, and then centrifugalized. The relative depressor action of equal quantities of these extracts, injected intravenously, were then determined on dogs under light ether anæsthesia. The extracts were always used within a few hours after they were prepared. They were kept on ice during the interval between the preparation and the injection. Of course we do not know that this treatment of the organs will yield any or all of the substances passed into the tissue lymph in normal activity, and we are practically certain that it yields substances not produced in normal activity. But it seemed to us that the Ringer's solution extraction would in all probability yield less artifacts than would more destructive treatment of the tissues. The active substance of the adrenals, as well as most of the ferments of the digestive gland, are secured in salt solution extracts of these organs. If our hypothetical vaso-dilation hormones in the tissues are in any way akin to these they ought to be secured in a Ringer's solution extract.

THE RELATIVE DEPRESSOR ACTION OF THE EXTRACTS FROM THE DIFFERENT ORGANS.

Our results are given in Table I. It will be seen that the greatest depression is produced by the organs having a large external secretion, such as the salivary glands, the pancreas and the mucosa of the stomach and the intestine. The lungs, kidney, liver, and spleen come next in order, while the thyroids, thymus, lymph glands, testicles, and skeletal muscles have practically no depressor action, when prepared according to our method. Three experiments with Ringer's solution extract of active mammary glands (dog) place the mammary glands in the group with the salivary glands and the pancreas. The lachrymal glands were not tested. The only organ so far tested that does not exhibit the above correspondence between depressor action and secretion is the brain. Nervous tissue has no external secretion, but a Ringer's solution extract of the fresh brain prepared as above produces a fall in the blood pressure as great or greater than the extracts of the salivary gland and the pancreas.

TABLE I.

Intravenous Injection of Ringer's Solution Extract of Equal Quantities of Fresh Organs from the same Animal. The Injections were made into Dogs under Light Ether Anæsthesia. The Figures for each Tissue represent the Average of Three Experiments, 10 c.c. of the Extract being injected each Time. Two or Three Dogs were used for the Injections in each Experiment.

No. of experiment	Fall of carotid blood pressure in mm. Hg.												
	Pancreas.	Intestinal mucosa.	Stomach mucosa.	Salivary glands.	Lung.	Kidney.	Spleen.	Liver.	Thyroid.	Thymus.	Lymph glands.	Skeletal muscle.	Testicle.
1	66	..	34	34	..	..	30	26	..	..	..	0	0
2	60	..	30	28	..	30	20	8	3	..	..	0	0
3	86	..	52	40	..	6	8	8	15	..	..	14	0
4	56	40	50	68	..	20	30	4	4	..	0	5	4
5	32	66	30	52	26	6	4	6	5	0	12	5	6
6	54	30	30	46	15	10	16	20	4	4	4	0	10
7	66	60	52	52	36	40	10	10	4	15	10	0	4
8	..	50	56	52	36	36	10	30	8	5	8	6	0
9	60	45	60	42	40	18	20	8	6	8	6	6	6
Average	60	48	44	46	31	21	16	13	6	6	6	4	3

THE RELATIVE DEPRESSOR ACTION OF EXTRACTS OF RESTING AND FATIGUED SALIVARY GLANDS.

In these experiments the submaxillary gland on one side was left at rest and the chorda lingual nerve on the other side stimulated with the tetanizing current intermittently for one and one-half to two hours, or until the gland had yielded 45–50 c.c. of saliva. The dog was under light ether anæsthesia during this period. The glands were then extirpated and ground up in washed sand, and extracted with equal quantities of Ringer's solution in the usual manner. The salivary glands were chosen because they are the most convenient to obtain in a state of rest and state of fatigue or exhaustion.

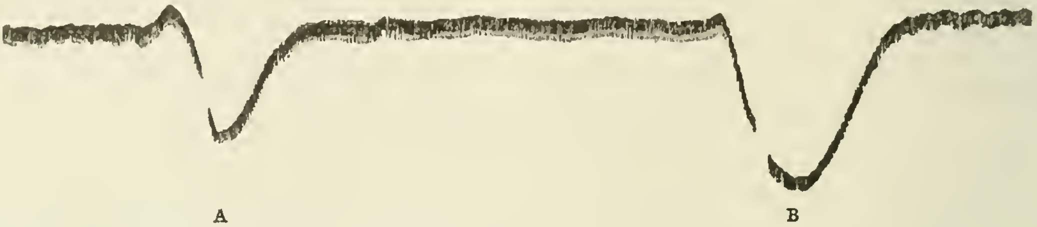


FIGURE 1. — Four sevenths the original size. Dog. Tracing of carotid blood pressure. Intravenous injections of 7 c.c. Ringer's solution extract of dog's submaxillary gland (fresh). *A*, extract of fatigued gland. *B*, extract of resting gland. Showing diminution of the depressor substance in the fatigued gland.

Our results are tabulated in Table II. The extracts of the fatigued glands show only one third the depressor action of the rested gland. The Ringer's solution extract of the fatigued glands contains much less mucin than that of the rested glands. But this difference in mucin content cannot account for the difference in action, because a Ringer's solution extract of the parotid gland (dog) produces as much depression as the extract of the submaxillary gland, although the former is practically free from mucin.

DOG'S SALIVA CONTAINS DEPRESSOR SUBSTANCES.

Intravenous injection of 10 c.c. of submaxillary saliva produces a temporary lowering of the blood pressure of the same type as the extract of the gland. It would thus seem that some of the constituents of the gland that yield depressor substances on extraction with

Ringer's solution pass into the saliva in ordinary physiological activity of the gland. These substances in so far as they pass into the saliva do not come in contact with the capillaries or arterioles and cannot therefore have any hemodynamic action.

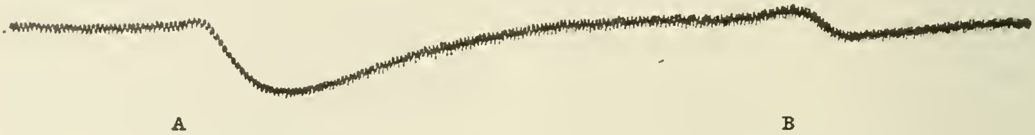
TABLE II.

THE DEPRESSOR ACTION OF RINGER'S SOLUTION EXTRACT OF THE RESTING AND THE FATIGUED SUBMAXILLARY SALIVARY GLANDS OF THE SAME DOG. THE GLANDS WERE EXTRACTED FOR THE SAME LENGTH OF TIME, WITH EQUAL QUANTITIES OF THE SOLUTION. EQUAL QUANTITIES OF THE EXTRACTS WERE INJECTED INTRAVENOUSLY. THE FIGURES GIVE THE FALL IN THE CAROTID PRESSURE IN MM. HG.

No. of experiment.	Fall of carotid pressure in mm. Hg.				
	Rested gland. Fall in bl. pressure.		Fatigued gland.		
			Fall in bl. pressure.		Remarks.
1	60 70 60 40	60	16 20 30 10	19	Intermittent tetanizing of c. t. nerve for 90′. 45 c.c. saliva.
2	70 60 80	70	10 12 10	11	Intermittent tetanizing of c. t. nerve for 80′. 50 c.c. saliva.
3	50 70 60 70 60	62	10 20 20 30 20	20	Intermittent tetanizing of c. t. nerve for 120′. 45 c.c. saliva.
4	80 60	70	6 10	8	Intermittent tetanizing of c. t. nerve for 120′. 50 c.c. saliva.
5	40 36 50	42	10 6 10	8	Intermittent tetanizing of c. t. nerve for 110′. 50 c.c. saliva.

THE NATURE AND THE POINT OF ACTION OF THE DEPRESSOR SUBSTANCES.

The fact that water or salt solution extracts of the adrenals yield the active principle of these glands, and that ferments of the pancreas and the gastric glands can be similarly extracted, seems to render it probable that at least some of the substances produced during normal activity of an organ are present in the organ extract as prepared in these experiments. But it seems even more probable that many substances not normally produced in the organ are also present in the extract. With the exception of the adrenals it is therefore not possible to say whether the hemodynamic action of an extract is due to physiological metabolites or to artifacts, or to both. Albumoses seem to be present in organ extracts in general. Extracts of stomach and intestinal mucosa may contain albumoses absorbed in the course of digestion. The pancreas extract may contain albumoses owing to digestive action of trypsin. But these possibilities do not apply to



FIRURE 2. — Two fifths the original size. Dog. Record of carotid blood pressure on intravenous injection of 4 c.c. of Ringer's solution extract of dog's submaxillary gland (fresh). *A*, extract of resting gland. *B*, extract of fatigued gland. Showing a decrease of the depressor substance in the fatigued gland.

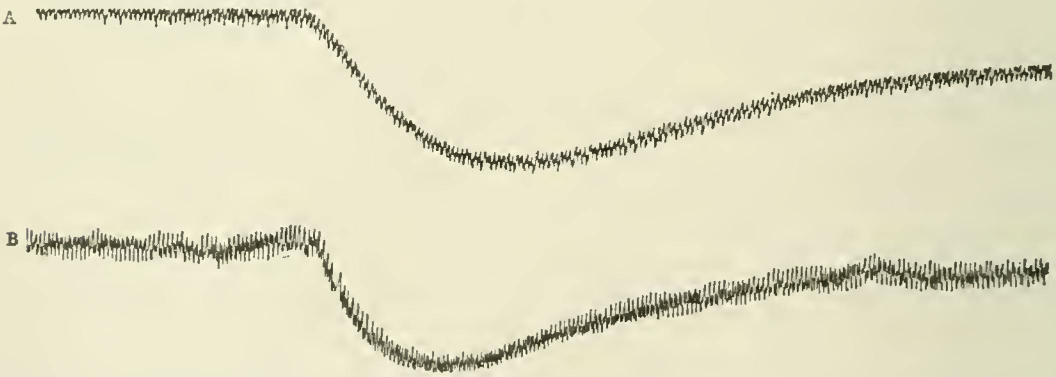


FIGURE 3. — Four sevenths the original size. Dog. Record of carotid blood pressure. Intravenous injections of 5 c.c. of Ringer's solution extract of dog's parotid gland (fresh). *A*, extract not boiled. *B*, extract boiled five minutes. Showing some destruction of the depressor substances by boiling.

such organs as the salivary, the lachrymal, and the mammary glands. The work of Lohmann,[10] von Fürth and Schwartz,[11] and others point to cholin as at least one of the depressor substances in the extracts of most tissues. This might account for the great depressor action of the salt solution extracts of nervous tissues. In the hands of most investigators, cholin, introduced intravenously, causes vaso-dilation. Modrakowski[12] claims, however, that pure cholin causes a rise instead of a fall of the blood pressure; and Popielski[13] concludes that neither albumoses nor cholin is responsible for the depressor action of organ extracts in general.

We can confirm the observations of previous workers as to the relatively great resistance of the depressor substance or substances to boiling. This applies also to the depressor action of saliva. Boiling the extract or the saliva diminishes its depressor action in all cases, but does not abolish it. This seems to point to a relatively simple substance such as albumose or cholin. The fact that the fatigued gland contains less instead of more depressor substances indicates that the depressor action is not due to *acid bodies* produced in activity.

The decrease in depressor substances in an organ *pari passu* with increasing activity and fatigue is so far the only direct evidence at hand in support of the view that in the depressor action of organ extracts we are dealing with hemodynamic substances actually involved in normal organ activity. In view of the probability that organ extracts contain more than one vaso-dilator substance, and that the number and character of these substances probably vary to some extent with the methods of extraction, it is premature to give them a specific name as has been done by Popielski.

All workers in this field seem to agree that the lowering of the blood pressure by small quantities of organ extracts is due to a direct action on the walls of the blood vessels rather than to an action on the heart, the vaso-motor or the vagi centres. The usual interpretation is that the depressor substance in the extract lowers the tonus of the musculature of the arterioles. Action on the capillaries is not considered. In our own experiments small quantities of the extracts did not weaken the heart appreciably, nor did atropin or sectioning of the vagi influence the results.

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[10] Lohmann: Archiv für die gesammte Physiologie, 1907, cxviii, p. 215.

[11] von Fürth and Schwartz: *Ibid.*, 1908, cxxiv, pp. 113, 261.

[12] Modrakowski: *Ibid.*, 1908, cxxiv, p. 601.

[13] Popielski: *Ibid.*, 1909, cxxviii, p. 191.

We are unable to offer any evidence as to the mode of action or point of action of the depressor substances. The organ metabolites or hemodynamic hormones may act on the arterioles or on the capillaries, or on both. If the action is on the arterioles we have the alternatives suggested by Gaskell and by Bayliss and Starling. According to Gaskell's view, the metabolites act on the musculature of the arterioles from the tissue side, and hence before reaching the blood stream. This hypothesis explains the localized action without having to assume a specificity of organ metabolites and organ blood vessels. The investigations in the last ten years of the mode of ending of the lymph vessels in the tissues render impossible the existence of the relation between the tissue lymph and the arteriole musculature demanded by Gaskell's hypothesis. *The tissue spaces that Gaskell deals with are the tissue spaces of the arteriole musculature and not those of the organ cells.* Even if these two systems of tissue spaces were not separated by septa or membranes the metabolites would pass toward the point of elimination, the capillary wall, rather than toward the walls of the arterioles. It must be admitted, however, that the transition, histological and physiological, from arteriole to capillary and from capillary to vein is probably a gradual one. If the capillaries are defined, physiologically, as the regions of the blood vessels mediating the exchange between the blood and the tissue lymph, the capillary area probably begins before the complete disappearance of the arteriole musculature and extends for some distance into the smaller veins, which would render Gaskell's hypothesis tenable in part.

If the depressor effect of the organ extracts is due to action on the arterioles, this action must be in accordance with the hypothesis of Bayliss and Starling, from the blood side and after the substances have passed through the endothelial cells.

Acting in the capillary area the tissue metabolites may increase the blood flow by (1) increasing the lumen of the capillaries; (2) by reducing the coefficient of external friction of the capillary walls.

The literature dealing with the contractility of the walls of the blood capillaries, apart from elasticity and mechanical tension, is reviewed in the recent paper by Steinach and Kahn.[14] Three types of changes in the capillary walls affecting the lumen of the capillaries have been described: (*a*) a bulging or pushing in towards the lumen of the spindle-shaped nuclei of the cells (Stricker, Tarschanoff, Golubeu, Severini); (*b*) variations in the thickness of the endothelial cell wall (Stricker, Biedl); (*c*) actual contraction of the endothelial cells (Stricker, Roy and Brown, Steinach and Kahn). We have not ourselves done any work in this field, but a review of the work of the observer just cited leaves the impression that the point best established is the actual or independent contractibility of the capillary walls. It seems to us wellnigh impossible to measure small variations in the thickness of the capillary walls in living tissue and under fairly normal physiological conditions. The mechanism of the contractibility is probably similar to that involved in amœboid movement. The above conception demands that with an organ at rest the capillary cells are in greater "tone," that is, they have greater thickness and are less extended. This tone is diminished in organ activity, and this, aided by the same or greater hydrostatic pressure in the lumen results in increased size of the capillary. One might readily formulate a hypothesis that would account for this change in "tone" by changes in the surface tension in the capillary cells, except for the fact that recent work seems to have rendered untenable the theory of surface tension variations as the essential factor in amœboid movements.

It is probable that external friction is a negligible factor in the resistance to the flow of the blood in the capillary region. With the low pressure and slow rate of flow the plasma film in actual contact with the surface of the endothelial cells must be practically stationary. But this does not apply to the friction of the blood corpuscles in contact with the capillary walls. There must be more or less contact of both erythrocytes and leucocytes with the capillary walls, even when the capillaries are of large enough calibre to allow relatively free passage of the corpuscles. But the capillary lumen may be so small that the erythrocytes are actually pressed out of shape in passage. Under such conditions the external friction must be much greater. The possibility of variations in the "adhesive property" or external friction of the endothelial cells with reference to the corpuscles is suggested by the phenomena of agglutination, and by the clogging of the capillaries by the corpuscles under pathological conditions. Both series of phenomena are undoubtedly due to changes in the surface of the cells through the action of substances in solution. But for the present the suggestion that organ activity results in lowering of the external friction of the capillary walls through metabolites or hormones, and hence an increased blood flow, must remain a mere speculation, as we see no way of proving or disproving it.

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[14] STEINACH and KAHN: Archiv für die gesammte Physiologie, 1903, xcvii, p. 105.

Since the viscosity of the blood is diminished by elevation of the temperature of the blood,[15] it might be inferred that this is a factor of the local increased blood flow in organ activity, since organ activity involves heat production and raises the temperature of the blood in the organ. But an increase of the CO_2 tension in the blood seems to increase the viscosity.[16] And in the case of the organs of external secretion the temperature factor may be more than counterbalanced by the concentration of the blood in passing through the capillaries — a concentration in corpuscles in every case, and in some cases also a concentration in the substances in solution. Thus, the blood coming from an active salivary gland has probably much greater viscosity than the blood going to the gland. If the viscosity or internal friction of the blood and the external friction on the part of the capillary walls were the only factors concerned in the increased blood flow in organ activity, there ought to be little or no increase in the volume of the active organ. This is contrary to the facts in the case.

Henderson[17] has recently advanced the hypothesis that the calibre of the capillaries is governed indirectly by the CO_2 content of the blood (and the tissue lymph) through the "tonus of the tissues." It is held that a decrease in the CO_2 tension increases the tissue tonus, and the capillaries are thus compressed mechanically, while an increase of CO_2 has the opposite action. As organ activity leads to an increased CO_2 tension in the tissue lymph and the blood, the mechanism pictured by Henderson may be a factor in the production of the increased blood flow in an organ in activity. It is not clear, however, that small variations in CO_2 tension produce these changes in "tissue tonus," especially in view of the work of Lee and others which indicates that CO_2 has a *primary stimulating action*, at least on muscle and nervous tissue. Bayliss found that an increased CO_2 tension in the perfusion fluid in frogs augments the blood flow, but he interprets this as a direct *vaso-dilator* action of the CO_2.[18]

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[15] Burton-Opitz: Archiv für die gesammte Physiologie, 1900, lxxxii, p. 447.

[16] Koranyi and Bence: *Ibid.*, 1905, cx, p. 513; Adam: Zeitschrift für klinische Medizin, 1909, lxviii, p. 177.

[17] Henderson: This journal, 1910, xxvii, p. 152.

SUMMARY.

1. The degree of depressor action of Ringer's solution extracts of fresh organs on intravenous injection is much greater in the case of organs having an external (watery) secretion than in the case of organs having no external secretion. This supports the hypothesis of a greater production of capillary dilator metabolites or hormones in organs producing an external (watery) secretion in view of the need of a greater water supply in the activity of these organs. Nervous tissue makes an exception to this rule.
2. The degree of depressor action of Ringer's solution extracts of fresh organs decreases with the increasing fatigue or exhaustion of the organ. This statement is based on experiments on the salivary glands. This fact supports the view that at least some of the hemodynamic substances in the Ringer's solution extracts of fresh organs are metabolites or hormones produced in normal activity. The depressor substances are relatively thermo stable. They appear to pass into the external secretion, in traces.
3. The data are discussed with reference to the probable mode of action of the hemodynamic metabolites on the blood capillaries.

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[18] BAYLISS: Journal of physiology, 1901, xxvi, p. xxxiii; Ergebnisse der Physiologie, 1905, v, p. 319.

STUDIES ON THE CIRCULATION IN MAN. — III. THE INFLUENCE OF FORCED BREATHING ON THE BLOOD FLOW IN THE HANDS.[1]

By G. N. Stewart.

[*From the H. K. Cushing Laboratory of Experimental Medicine, Western Reserve University.*]

IN connection with some experiments on the influence of inhaling oxygen on the blood flow in the hand in a case of cyanosis, in which apparently the cyanosis was not associated with any change in the respiratory movements, it was found necessary to know how the blood flow was affected by voluntary increase in the depth and frequency of the respirations. Experiments were made for this purpose on two normal persons. The hands being inserted into the calorimeters as described in previous papers, the amount of heat given off was determined for a sufficient period with ordinary breathing. Then the subjects were instructed to breathe more rapidly and deeply and the heat exchange again determined for a given period of forced breathing. A period of ordinary breathing succeeded, and then, perhaps, another period of forced breathing. The forced breathing was never carried to the point of discomfort, as the object of the experiments was to determine the effect of a moderate increase in the pulmonary ventilation. The subjects were therefore warned that great efforts to accelerate and deepen the breathing were not to be made. The result is all the more striking. For in both cases a very distinct diminution in the flow through the hands was observed during the periods of increased respiration. The change is not exactly synchronous with the alteration in the respiration, as is shown by inspection of the minute readings in the protocols cited.

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[1] Paper I, "The measurement of the blood flow in the hand," and Paper II, "The effect of reflex vasomotor excitation on the blood flow in the hand," were sent to Heart, 1911, ii. A preliminary account of the method of measuring the blood flow in the hand was communicated to the American Physiological Society, December 29, 1910 (Stewart: This journal, 1911, xxvii, p. xx), and "A comparison of the blood flow in the hands in a case of birth palsy and a case of infantile paralysis" was communicated to the Society for Experimental Biology and Medicine, December 21, 1910 (Stewart: Proceedings of the Society for Experimental Biology and Medicine, 1910, viii, p. 43).

M. C. Age, 23. Weight, 146 pounds (stripped). Height, 5 ft. 10 inches. Mouth temperature, 36.45°. Rectal temperature, 37.4°. Pulse, 88 (sitting).

3.45.30 P. M. Hands put into bath at 30.1°.

3.55.30 P.M. Hands put into calorimeters, right into A, left into B. 3050 c.c. of water in each calorimeter.

Time.	A	B	Notes.	Time.	A	B	Notes.
3.58	29.91	29.91	Ordinary breathing.	4.16	31.22	31.22	Room 23.0
3.59	30.02	30.00		4.17	31.26	31.25	At 4.17 ordinary breathing resumed.
4.00	30.13	30.12	Room 23.9.	4.18	31.30	31.32	
4.02	30.33	30.31	Room 22.8.	4.19	31.34	31.36	
4.05	30.58	30.53		4.20	31.39	31.41	Room 23.3.
4.06	30.67	30.63		4.21	31.44	31.43	
4.07	30.74	30.72		4.22	31.47	31.50	
4.08	30.80	30.78	At 4.08 began deeper and faster breathing.	4.23	31.53	31.55	Room 23.2.
4.09	30.87	30.84		4.24	31.60	31.63	
4.10	30.92	30.91					
4.11	30.98	30.96	Room 23.4.	4.26	31.73	31.75	Room 22.7.
4.12	31.04	31.03		4.27	31.79	31.83	
4.13	31.09	31.10	Room 23.2.	4.28	31.85	31.89	At 4.29 hands taken out of calorimeters. Room 23.0.
4.14	31.14	31.13		4.29	31.91	31.94	
4.15	31.18	31.19	Room 22.9.	4.44	31.72	31.75	

Volume of right hand 475 c.c., of left 460 c.c.

Taking the temperature of the arterial blood at the wrist as 36 9 (*i. e.*, 0.5° below the rectal temperature, which was the difference actually found in this subject in experiments specially made to determine the difference between the rectal temperature and that of the arterial blood coming to the hand), we get for the first period of ordinary breathing (ten minutes) a flow of 60.13 gm. of blood per minute for the right hand and 58.69 gm. for the left (with mean calorimeter temperatures of 30.35 and 30.34 respectively), *i. e.*, 12.66 gm. per 100 c.c. of hand per minute for the right hand and 12.76 gm. for the left. For the first four minutes of forced breathing the flow comes out 47.28 gm. per minute, or 9.95 gm. per 100 c.c. per minute for the right hand, and 48.5 gm. per minute, or 10.54 gm. per 100 c.c. per minute for the left hand. For the next five minutes of forced breathing the corresponding numbers are 37.99 and 7.99 gm. for the right hand, and 37.77 and 8.21 gm. for the left. For the whole nine minutes of forced breathing the flow is 42.08 gm. per minute, or 8.86 gm. per 100 c.c. per minute for the right hand, and 42.52 gm. per minute, or 9.24 gm. per 100 c.c. per minute for the left hand. For the first four minutes of the subsequent period of ordinary breathing the flow is 40.39 gm. per minute, or 8.50 gm. per 100 c.c. per minute for the right hand, and 40.19 gm. per minute, or 8.73 gm. per 100 c.c. per minute for the left. For the next eight minutes of ordinary breathing the corresponding numbers are 55.88 and 11.76 gm. for the right hand, and 56.76 and 12.34 gm. for the left hand, almost as much as in the first period of ordinary breathing. It will be seen that while the flow is diminished promptly by forced breathing, the change takes some minutes to reach its maximum. When natural breathing is resumed the flow increases, but again an interval measured by minutes is required for the maximum flow to be attained. The maximum diminution of the flow by the forced breathing (during the last five minutes of the period) is nearly 36.8 per cent for the right hand and 35.6 per cent for the left. For the whole period of forced breathing the diminution is 30 per cent for the right hand and 27.6 per cent for the left.

In the other normal person investigated (C. B.) the same general result was obtained. But it will be instructive to cite a protocol for two reasons: (1) The normal flow in the hands of this person is much less than in the case of M. C. He belongs to the group of normal persons whose hands are habitually cool or cold,[2] while M. C. belongs to a group with habitually warm hands. (2) C. B. forced the breathing to a greater extent than M. B., who was suffering from a slight cold. This probably accounts for the greater promptitude with which the maximum change was reached in the case of C. B. In neither case, however, was the exaggeration of the respiratory movements excessive.

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[2] STEWART: Cleveland medical journal, May 1, 1911, p. 391.

C. B. Age, 22. Weight, 130 pounds (with clothes). Height, 5 ft. 8½ inches. Mouth temperature, 36.4. Pulse, 78 (sitting). He says his hands always feel cool. His work, that of a laboratory assistant, does not differ materially from that of M. C. He had been in the open air on a cold morning for two or three minutes before the experiment was begun. Before that, he had been for a considerable time indoors.

11.28.30 A. M. Hands put into bath at 29.8°. Room, 18.9°.

11.41.40 A. M. Hands put into calorimeters, right into A, left into B. 3050 c.c. water in each (see table on p. 194).

For the first period (of sixteen minutes) of ordinary breathing the flow comes out 18.60 gm. per minute, or 4.53 gm. per 100 c.c. of hand per minute for the right hand, and 14.13 gm. per minute, or 3.40 gm. per 100 c.c. per minute for the left hand. For the succeeding period of forced breathing (six minutes) the flow is only 10.39 gm. per minute, or 2.43 gm. per 100 c.c. per minute for the right hand, a diminution of over 44 per cent. For the left hand the corresponding numbers are 8.40 gm. and 2.02 gm., a diminution of 40.6 per cent. The decrease is already apparent in the first minute. For the first two minutes after the resumption of ordinary breathing the flow for the right hand is 13.3 gm. per minute, or 3.1 gm. per 100 c.c. per minute, and practically the same for the left. The increase, accordingly, has begun, but it becomes greatly accelerated in the next five minutes of ordinary breathing, the flow rising to 24.11 gm. per minute, or 5.65 gm. per 100 c.c. per minute for the right hand and to 20.36 gm. per minute, or 4.90 gm. per 100 c.c. per minute for the left hand. For the immediately succeeding period of forced breathing (six minutes) the flow falls to 11.47 gm. per minute, or 2.69 gm. per 100 c.c. per minute for the right hand, a diminution of over 52 per cent, and to 12.39 gm. per minute, or 2.98 gm. per 100 c.c. per minute for the left, a diminution of over 39 per cent. It ought to be pointed out that with such small flows as those seen in this subject, particularly after a long immersion, slight variations in the calibre of the vessels due to uncontrolled vasomotor changes will cause relatively great effects on the blood

Time.	A	B	Notes.	Time.	A	B	Notes.
11.39	29.29	29.34		12.07	29.62	29.54	Room 20.0.
11.43	29.24	29.32		12.08	29.65	29.56	
11.45	29.31	29.34	Room 19.8.	12.09	29.69	29.61	He says hands feel a little warmer than before starting deep breathing.
11.47	29.34	29.37		12.10	29.72	29.63	
11.48	29.37	29.39		12.11	29.74	29.64	
11.50	29.38	29.38		12.12	29.76	29.65	At 12.12 started deeper and faster breathing again.
11.51	29.39	29.39		12.13	29.77	29.65	
11.52	29.39	29.40		12.14	29.78	29.66	
11.53	29.42	29.42		12.15	29.79	29.67	
11.54	29.47	29.44	Room 19.9.	12.16	29.79	29.68	
11.55	29.49	29.45		12.17	29.80	29.69	
11.56	29.50	29.45		12.18	29.80	29.70	At 12.18 resumed ordinary breathing.
11.57	29.52	29.46		12.19	29.81	29.69	
11.58	29.54	29.51		12.20	29.83	29.71	
11.59	29.57	29.51	At 11.59 began deeper and faster breathing.	12.21	29.85	29.72	
12.00	29.57	29.51		12.22	29.86	29.73	
12.01	29.59	29.52		12.23	29.88	29.74	
12.02	29.59	29.52		12.24	29.90	29.75	
12.03	29.59	29.51		12.25	29.92	29.76	
12.04	29.59	29.52		12.26	29.92	29.76	Hands taken out of calorimeters at 12.26.
12.05	29.60	29.52	At 12.05 res'm'd ord'n'y br'th'g.	12.39	29.74	29.60	Room 20.6.
12.06	29.61	29.53		12.49	29.61	29.45	Room 19.7

Volume of right hand 427 c.c., of left 415 c.c.

flow. For this reason the differences between the two hands have little significance in this case. For the same reason the comparatively small increase in the flow in the right hand during the final period of ordinary breathing (to 16.94 gm. per minute, or 3.97 gm. per 100 c.c. per minute) and the absence of any sensible increase in the left hand (for which the corresponding numbers are 12.36 and 2.95) does not at all invalidate the conclusions deduced from the earlier observations in this experiment. The progressively increasing vaso-constriction in both hands, but especially in the left, has simply masked the increase conditioned by the return to ordinary respiration.

The cause of the diminution in blood flow produced by forced respiration is without question in part a mechanical effect on the action of the heart due to the changes in the intrathoracic pressure. Hill and Flack [2] have stated that the left ventricle becomes smaller, as shown by Roentgen ray pictures, the radial artery emptier, and the arterial blood pressure lower with each forced thoracic inspiration. That a chemical change may also be concerned is indicated by experiments on the case of cyanosis already mentioned, in which oxygen inhalation distinctly increased the blood flow in the hand without affecting the respiratory movements or the total pulmonary ventilation, while in normal persons it had no such effect.[3] It may be that the washing out of the carbon dioxide by the forced respiration causes, even in such short experiments and with such moderate exaggeration of the respiratory movements, those changes in the distribution of the blood, particularly its accumulation in the great veins, which Henderson [4] associates with "acapnia," and which, according to him, are so important a factor in surgical shock. Other vascular changes, for example, a diminution of the flow in the coronary circulation due to the fall of pressure in the aorta, which aid the mechanical changes in the thorax in decreasing the average output of the heart, may occur. Changes produced through the vasomotor centre on the peripheral vessels may also play a more direct part. This is at any rate suggested by the fact that the percentile change in the flow caused by forced breathing is not the same for the two hands as it might be expected to be were the whole effect a cardiac one. However, it is not intended to lay much stress on this suggestion, for the initial differences in the flow in the two hands are not sufficiently great to permit without hesitation the application of this criterion.[5] The beneficial influence of oxygen on the flow in the case of cyanosis may be explained as the result of the oxygen action in diminishing the excitability to carbon dioxide of the vasomotor centre and other mechanisms affected by hypercapnia. In this patient the partial pressure of carbon dioxide in the alveolar air was found by my colleagues, Drs. Macleod and Hoover, to be persistently much above the normal. The amplitude of the radial pulse increased distinctly during oxygen inhalation. The increase was easily detected by the finger, and was shown quite clearly on the sphygmograms.

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[2] Hill and Flack: Journal of physiology, 1910, xl, p. 347.

[3] See Paper IV of this series, Journal of pharmacology and experimental therapeutics, 1911, ii, No. 6.

[4] Y. Henderson: This journal, 1910, xxvii, p. 152, etc.

[5] Stewart: Journal of pharmacology and experimental therapeutics, *loc. cit.*

THE RELATION OF STIMULATION AND CONDUCTION IN IRRITABLE TISSUES TO CHANGES IN THE PERMEABILITY OF THE LIMITING MEMBRANES.

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I. Introduction.

THE conception of stimulation, as a physiological process, may relate to the entire organism or to an individual tissue or even cell. It is in its latter application that I propose to consider it in the present paper. Any irritable cell or tissue — as a protozoön, a gland cell, or an isolated muscle or nerve — may be suddenly aroused from an apparently complete quiescence to activity, or from a less to a more active state, by certain changes in its surroundings. These changes, physically considered, may be of the most diverse kind and yet evoke in the living system the same characteristic change of activity. We speak of the change in the environment as the stimulus, of that in the cell or tissue as the reaction or response. The latter is, qualitatively at least, constant and distinctive for any cell or tissue; it may involve a transformation of energy many times greater than that of the stimulus. It is characteristic of irritable tissues — though not exclusively so — that there is from the energetic point of view no constant and definable relation between stimulus and response: the ratio, energy transformation of stimulus to energy transformation of response $\left(\frac{dW_s}{dW_r}\right)$, varies within the widest limits, not only for different cells and tissues, but for the same tissue under different conditions. Hence a rigid and all-inclusive brief definition of stimulation is difficult to formulate. Thus a change in the direction of *increased* activity is not the only response possible to an irritable tissue: quite as often the change may be in the opposite direction: we then speak of inhibition;

but it is impossible to consider the two processes apart from each other, and any adequate theory of stimulation will necessarily involve or imply one of inhibition. In fact, the entire nature of the give and take, energetic and material, between living system and surroundings is the question at issue. In its most general aspect the problem may be proposed thus: through what means do changes in the environment influence the "vital" processes in the cell?

This question is almost as broad as biology itself. What we actually observe is that the vital processes change, showing sometimes increased, sometimes decreased activity, as the character of the environment changes. Study of the behavior of a given cell or tissue under changing external conditions discloses a certain constant correspondence or parallelism between the two series of changes. Although in their energetics and in the character of their processes it would be difficult to imagine a more complete contrast than that between, *e. g.*, the pinching of a nerve and the contraction of the muscle, the connection is nevertheless perfectly definite and constant. The scientific problem to be solved relates to the nature and conditions of this constant interconnection.

It is at once evident that the response, even where outwardly inconspicuous as in nerve, is an event of much more complex nature than the stimulus. If any such response, *e. g.*, a muscular contraction, is analyzed in detail, it appears as a series of separate processes succeeding one another in definite order, and of which each is the determining condition of that (or of those) immediately succeeding; these simpler processes may be called the serial components of the entire response; they may be symbolized by the series: *a* ... *b* ... *c* ... *d* ....[1] Of these the first (*a*) or that immediately due to the external change, is the determining condition of all the others; it is the primary or critical event of stimulation, the *Anlass*,[2] the first change in the living system as distinguished from the change in the non-living surroundings. The re-

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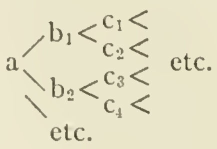
[1] Or perhaps better, since each event in the series may originate several:



[2] *Cf.* OSTWALD's Vorlesungen über Naturphilosophie, 2d edition, p. 299.

sponse, considered as a whole, is constant in its nature or quality — though it may vary quantitatively — for a given tissue; hence the initial or determinative process, *a*, must be constant in its essential nature. The question now to be considered is: what is the nature of this primary change, and why may it be called forth by such physically diverse agencies as heat, light, electricity, chemical, osmotic, or mechanical action, all of which may produce the same effect in an irritable tissue?

First, as to the seat of this change: it is obvious that events in the external world must first affect the surface of the living cell. But it is not evident that an effect confined on its first production to the surface must necessarily influence the processes in the interior. Conceivably the plasma membrane may simply play the part of a protective or insulating layer, while the distinctively irritable mechanism is situated within, — in the "living" protoplasm; and in fact this seems to have been the general or tacitly accepted belief until recently. There is, however, now ample evidence that the plasma membrane is far from being a mere partition or passive barrier between living substance and environment. It is rather to be regarded as the essentially irritable part of the cell, and as playing the rôle of a sensitive intermediary between the external world and the living protoplasm, so that alterations of the membrane, particularly in its permeability, necessarily involve changes in the metabolism and hence in the energy production of the cell.

This conviction has been reached gradually as the outcome of widely different investigations and points of view. Studies of the plasmolytic action of solutions and of the partition of dissolved substances between living cells and their media have shown that the plasma membranes of typical resting cells — like blood corpuscles or voluntary muscle cells — while freely permeable to water are during life almost if not quite impermeable to the majority of neutral salts and indifferent non-electrolytes, such as the sugars, polyhydric alcohols, and amino-acids. The protoplasm is thus sharply delimited from its surroundings by a surface film which in spite of its extreme thinness is apparently less permeable than the most impermeable of the precipitation membranes thus far known.[3] This peculiarity renders it possible for the enclosed protoplasmic complex, consisting as it does largely of easily diffusible crystalloid substances, to differ absolutely from its surroundings and to maintain a distinctive and highly complex composition, unlike that of any other known physico-chemical system. The sharp discontinuity between the cell and its medium is thus conditional upon the impermeability of the physical boundary between the living substance and its non-living environment. This impermeability is also the condition of other and equally important peculiarities. Phase boundaries in general are the seat of electrical forces. The two surfaces of the membrane — considering it as of a certain thickness — adjoin different solutions: each surface is thus to be regarded as the probable seat of a potential difference; since the two will in all probability differ, the existence of a potential difference between the exterior and the interior of the cell is to be assumed. The degree of this potential difference will depend on the nature and concentration of the ions at the boundaries and on the ionic permeability of the membrane. Now such potential differences have long been known to exist in irritable tissues; and, as will be seen below, not only the osmotic but also the electrical properties of the cell receive consistent explanation on the modern theory of solutions when the nature of the plasma membrane is taken into account. Since the sensitivity of irritable tissues to the electric current, and the production of electrical currents by such tissues during stimulation, are perhaps their most distinctive and constant characteristics, it follows that the plasma membranes, as the probable seat of the electrical changes accompanying stimulation, are also to be regarded as the most probable seat of the essential or critical event in the stimulation process.

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[3] *Cf.* Walden's investigation of the comparative permeability of precipitation membranes: Zeitschrift für physikalische Chemie, 1892, x, p. 699.

It is important to note that the impermeability indicated by plasmolytic or partition methods is to be regarded not as absolute or invariable, but as more especially characteristic of cells during the relatively inactive or resting periods of their existence. During activity the conditions are changed; obviously the entrance of food materials and salts into the cell is incomprehensible unless periods of increased permeability are assumed; and there is in fact abundant evidence that changes in the permeability of membranes play a fundamental rôle in vital processes: witness, to cite one striking example, the dissolution and re-formation of the nuclear membrane during mitosis. The facts and considerations to be brought forward below indicate that stimulation is also a process in which changes of permeability are fundamentally concerned. During this process the permeability of the plasma membrane apparently undergoes a sudden and marked increase; the state of increased permeability is temporary in normal stimulation, and on cessation of the stimulus the former semi-permeability is at once regained.[4] This semi-permeability is the normal condition of equilibrium during life, and in such a tissue as voluntary muscle or nerve it returns very rapidly after stimulation; hence any change of permeability is difficult to demonstrate in these tissues by direct methods (as by using dyes, etc.). The electrical variation or action current, as interpreted on the membrane theory, is the only index of altered permeability in such cases. But in the contractile tissues of certain invertebrates and in the osmotic motor mechanisms of sensitive plants an increase in permeability during stimulation is clearly demonstrable, as I shall shortly point out.

Before presenting direct evidence of an increased permeability during stimulation, it seems desirable to consider briefly the theoretically possible relations which the semi-permeable membranes of the irritable elements may bear both to the possibility of electrical stimulation and to the production of the characteristic electrical variation. So far as external evidence shows, these are the most constant peculiarities of irritable tissues, and indeed in some cases, as in nerve and the electric organs, the only ones directly observable. The essential conditions of these changes are therefore in all probability those of the stimulation process itself.

II. THEORETICAL.

The first unequivocal evidence that the semi-permeable membranes of the irritable elements are the seat of stimulation was brought forward by Nernst in 1899.[5] In its relation to the electrical current any irritable living tissue is to be regarded as an electrolytic conductor subdivided by semi-permeable membranes. A current traversing such a system can produce decided changes only at the semi-permeable partitions, *i. e.*, the plasma membranes of the cells or elements, where the movement of ions is blocked or checked. The current carries ions to the membrane, producing increased concentration of cations on the side directed toward the anode and of anions on the opposite side; this tendency to increased concentration at the membrane is opposed by diffusion, which tends to equalize the concentration gradients thus arising. On the assumption that stimulation requires a certain critical increase in the concentration of ions *at* the membrane, Nernst showed, by a consideration of the conditions at a single membrane, that there should be a relation between the duration of a given current and its stimulating power, of such a kind that the relation, $i\sqrt{t} = c$, should hold, where i is the intensity, t the duration, of the current, and c a constant corresponding to the threshold of stimulation. This relation was first experimentally tested by Zeynek, for the case of alternating currents; the relation $i/\sqrt{n} = c$ (n being the rate of alternation) was found to hold for sensory nerves within a wide range in the rate of alternation; Nernst and Barratt showed the same for the motor nerves of the frog, and other observations (Weiss, v. Kries, Reiss, Lapicque, Lucas) have shown good agreement with theory within a certain range of durations. The formula in its original simple form is, however, not entirely adequate, especially for longer durations of the current, since one of its implications is that any current, no matter how weak, will stimulate if its duration is sufficient; it also fails to account for certain characteristic peculiarities of electrical stimulation, such as the relation of the stimulating effect to the rate of change in the stimulating current — *i. e.*, the fact that slowly increasing currents do not stimulate — and the law of polar stimulation. Hence modifications have been introduced, particularly by Lapicque and Hill, which, without essentially altering the principle, have greatly increased the range of its application.[6] Hill in particular has pointed out that it is more in accordance with the histological facts to consider the case of two membranes close together; and he has derived a formula by the use of which the calculated relation between the duration and the stimulating intensity of a current is found to show an astonishingly close agreement with observation.[7] There can therefore be little doubt of the essential correctness of Nernst's point of view, and we may with confidence regard the semi-permeable membranes of an irritable tissue as the seat of electrical stimulation.

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[4] Of course a time factor enters here, characteristic for different tissues. The period of increased permeability resulting from momentary stimulation appears to correspond in its duration to the refractory period. This varies in the frog from .001 second or less in nerve to more than a second in cardiac muscle. These durations closely correspond to those of the respective action currents. See below, p. 221.

[5] NERNST: Nachrichten von der königlichen Gesellschaft der Wissenschaften zu Göttingen, Math.-physik. Klasse, 1899. Republished in Archiv für die gesammte Physiologie, 1908, cxxii, p. 275.

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[6] LAPICQUE: Journal de physiologie, 1907, ix, pp. 565, 620, 1908, x, p. 601, 1909, xi, pp. 1009, 1035; HILL: Journal of physiology, 1910, xl, p. 190.

What is the nature of the process occurring at the membrane? The most constant accompaniment of stimulation is the electrical variation known as the action current; the potential difference between the external surface and the exposed interior of the muscle cell or nerve fibre undergoes a sudden and reversible decrease during stimulation. What is the relation of the membrane to this change?

The question of the nature and significance of the bioelectric processes and of their relation to the membranes of the tissue evidently arises here. Those known structural and physico-chemical peculiarities of the living tissue which can serve as the sources of potential differences must therefore be first considered. From this standpoint the muscle or nerve appears essentially as a solution containing electrolytes and subdivided by semi-permeable colloidal membranes; it is also to be noted that the electrolyte content of the surrounding medium is typically different from that of the cell. Contact differences of potential must necessarily arise between the adjoining solutions in such a system. But by taking into account simply the nature and concentrations of the electrolytes inside and outside the cell and proceeding on the assumption that the ionic migration rates are the same as in simple aqueous solutions, it has proved impossible to account for the observed conditions; thus the potential differences in the concentration chains studied by Oker-Blom,[8] — even with $n/10$ lactic acid as the solution corresponding to the electrolyte within the cell, — are much less than those found in a single muscle, where the demarcation current potential may approximate 0.1 volt — not to speak of the case of electric organs where potential differences of apparently hundreds of volts may exist. Some condition which shall more or less permanently prevent the tendency to equalization of differences of ionic concentration seems required; and as Ostwald first pointed out in 1890 the semi-permeable plasma membranes seem to furnish the structural arrangements needed.[9]

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[7] *Cf.* K. LUCAS: Journal of physiology, 1910, xl, p. 225.

[8] OKER-BLOM: Archiv für die gesammte Physiologie, 1901, lxxxiv, p. 191.

[9] OSTWALD: Zeitschrift für physikalische Chemie, 1890, vi, p. 71.

It can readily be demonstrated that membranes influence the rate of ionic diffusion and differently for different ions; certain membranes (as copper ferrocyanide) may completely prevent the passage of certain ions. If a membrane has selective permeability such that only one class of ions, *e. g.*, the cations of a given electrolyte, may pass but not the accompanying anions, the potential difference between two solutions of the electrolyte may be greatly increased by interposition of such a membrane. Furthermore a condition then arises which permits the summation of the potential differences of adjacent elements, as in a battery of galvanic cells in series. Brünings has shown experimentally that such summation of the demarcation current potentials is possible for frog's muscle,[10] and has adopted in explanation Ostwald's suggestion of a selective ionic permeability (permeability to cations but not to anions) of the plasma membrane. The high potential differences found in electrical organs may thus be accounted for; in fact the structural arrangements found in these organs are in their essential features such as might have been predicted from this theory. Thus the hypothesis that the plasma membranes are the seat of the electromotive properties of irritable tissues arises for consideration.

It may be said that virtually all students of electrophysiology are now agreed in attaching fundamental importance to the plasma membranes. It is not yet quite clear how the relation of these structures to the observed potential difference between outside and inside of the cell (demarcation current potential) is best to be regarded. It is possible to consider the plasma membrane as a distinct solid phase of appreciable thickness in contact with two fluid phases, the protoplasm and the external medium (lymph, etc.), each phase having its characteristic composition, with corresponding solvent, dissociative, and other properties. The character and concentration of the ions in the membrane will necessarily differ from those in the media adjoining; hence a potential difference will arise at each boundary; and the potential difference between internal protoplasm and outer medium will be equal to the difference between these two. Or the membrane may be regarded as simply a partition of negligible thickness, pervaded by the solvent, and acting simply by influencing the diffusion rates of the ions in the two adjacent media — *i. e.*, as more or less freely permeable to the different ions. The second point of view is simpler, and may be regarded as a special case of the first, so that we shall adopt it in our consideration. Assuming, then, the importance of the membrane, the chief questions become: (1) what are the electrolytes concerned? and (2) what is the character of the membrane?

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[10] Brünings: Archiv für die gesammte Physiologie, 1903, xcviii, p. 241.

From the experimental side the most illuminating investigations have undoubtedly been those of Cremer and of Haber and Klemensiewicz.[11] These have shown that thin films of water-soaked glass and layers of benzol — media which may be briefly characterized as permeable only to water and the ions of water — may, when in contact with weak solutions of acid or alkali, be the seat of very considerable potential differences, amounting to several tenths of a volt for concentrations of a few thousandths normal. The remarkable feature of such elements is their sensitivity to changes in the hydrogen or hydroxyl ion concentration in the neighborhood of the neutral point. This feature depends on the general facts (1) that it is the ratio and not the absolute concentrations of the ions in the adjoining phases which determines the potential difference, and (2) that the H-ion concentration in the non-aqueous phase is low and essentially constant. Changes in hydrogen-ion concentration corresponding to only a few hundred thousandths normal may thus produce potential differences comparable to those found in living tissues; in fact, assuming similar properties in the plasma membranes, carbon dioxide in the concentrations formed in metabolism is sufficient to account for the potential differences observed in tissues. It is to be emphasized that these conclusions are based not only on theoretical deduction but also on experimentally determined fact. The possibility that the cation concerned in the phenomena of demarcation and action currents is no other than the hydrogen ion must be regarded as experimentally substantiated. The plasma membrane in order to act like the membranes studied by Haber and Klemensiewicz must have peculiar solvent properties; it must be freely permeable to water and the ions of water, but difficultly or not at all to other ions. Of course these are limiting conditions which are probably not fully realized by many existing plasma membranes — except perhaps those of electrical tissues, where the insulation, *i. e.*, the impermeability of the membranes to one class of ions (probably anions), must be very complete. For muscle and nerve it seems sufficient to assume that H^+ and OH^- ions pass readily, but others with difficulty.

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[11] Cremer: Zeitschrift für Biologie, 1906, xlvii, p. 562; Haber and Klemensiewicz: Zeitschrift für physikalische Chemie, 1909, lxvii, p. 385.

The circumstance that the fluids bathing the tissues of higher organisms are virtually neutral in reaction and contain carbonates and phosphates in such proportions as automatically to conserve neutrality acquires new significance from this point of view. Slight quantities of acid produced within the cell near the membrane might thus, with a neutral external medium, give rise to considerable differences of potential; the conditions are apparently such that the carbonic and other weak acids produced in metabolism may well be the source of the demarcation current potential. It has been held that an automatic conservation of neutrality is equally characteristic of the living protoplasm; but this view is contradicted by the fact that muscle on tetanization, and particularly after heat rigor, chloroform rigor, and similar changes, becomes decidedly acid. The facts indicate that while there can be little change in the neutrality of the blood plasma or lymph, the reaction of the protoplasm is subject to considerable variation, though the range of this variation may have its limits. Since there is always oxidation in progress within the cell, we may infer that the normal H-ion concentration of the protoplasm, while low, is yet considerably higher than that of the adjacent lymph. Under these conditions a potential difference of the kind observed, with outer surface positive, will exist between the exterior and interior of the cell.

We assume therefore that the cations concerned in the production of the demarcation current potential are H-ions derived from the dissociation of carbonic and other weak acids produced in metabolism. The other electrolytes present in the cell seem not to answer to the requirements. Thus potassium, which in vertebrata has a higher concentration in the muscle cells and nerve fibres than in the blood plasma, cannot be the cation, for increasing its concentration in the external medium to an equality with that in the cell does not annul the potential difference, as Höber [12] has shown. Again, the production of the action current, on the membrane theory, must mean an increased loss of the electrolyte from the cell; now, while this is true for carbon dioxide during activity, it is not for potassium or any other salts, so far as has been observed. We may infer with a high degree of probability that carbonic acid is the chief, though perhaps not the sole, electrolyte concerned in the production of the demarcation current potential.

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[12] *Cf.* Höber: Physikalische Chemie der Zelle und der Gewebe, 1906, p. 316.

The above considerations relate particularly to the conditions of the demarcation current. They indicate that the plasma membrane of the resting cell is to be regarded during life as the seat of a potential difference, in other words, as being electrically polarized in such a sense that the solution in contact with its outer face is positive relatively to the enclosed protoplasm. During stimulation this potential difference decreases; we have now to inquire into the possible and probable conditions of this change.

Briefly restated, the demarcation current potential on the present theory is to be regarded as a contact potential of a peculiar kind, where the ions of the contiguous solutions are modified in their velocity by an interposed membrane. According to Nernst's theory, the potential difference between two unequally concentrated adjoining solutions of the same electrolyte depends on the ratio between the concentrations of the dissociated part in each solution and on the difference between the ionic velocities. The formula expressing this relationship is:

$$E = \frac{RT}{F} \frac{u - v}{u + v} \, ln \, \frac{c_2}{c_1}.$$[13]

If we have interposed between the solutions a thin partition which is freely permeable to the cation but impermeable to the anion, the formula becomes $E = \frac{RT}{F} \, ln \, \frac{c_2}{c_1}$, since v, the velocity of the anion, is reduced to zero. This formula, however, is identical with that describing the conditions at any ion-liberating surface, such as a metallic plate in a solution of its salt, where c_2 represents the external ionic concentration at which the potential difference would become zero — which, expressed in terms of osmotic pressure, is equal to the specific solution tension of the metal — and c_1 the existing concentration of the metallic ions in the solution. Such a membrane, in other words, would act precisely like any ion-yielding surface of a solution tension corresponding to c_2. This, however, would be the case only so long as the membrane remained impermeable to the anion; with any increase in permeability sufficient to allow the anions to pass v acquires a positive value and $\frac{u - v}{u + v}$ becomes less than unity, a change involving decreased value of E; the degree of this fall in potential will evidently depend on the relative migration rates of the ions through the altered membrane. Evidently also the value of E would decrease with decrease in the ratio $\frac{c_2}{c_1}$ independently of any change in ionic velocity. Which of these conditions is realized in stimulation?

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[13] In this formula E is the potential difference in volts, R the gas constant, T absolute temperature, u velocity of cation, v of anion, ln natural logarithm, c_2 the concentration of the dissociated part of the more concentrated, c_1 of the more dilute solution, F the Faraday constant. The ions are regarded as monovalent.

With an electrolyte having a relatively rapid cation, like an acid, the more dilute solution (c_1) becomes positive. In the case of the living cell the external solution is positive relatively to the interior; this condition corresponds to the higher concentration of carbon dioxide within the cell; the external medium in higher animals is in fact automatically kept neutral, *i. e.*, has an H-ion concentration of *ca.*10^{-8} normal at 25°. If we assume unchanged ionic velocities, *i. e.*, unaltered membrane, during stimulation, $\frac{c_2}{c_1}$ becomes the variable part of the above formula. It is clear that a fall of potential would on this assumption correspond to a *decrease* in c_2, the H-ion concentration within the cell, since c_1 is invariable or is only slightly and secondarily changed by the stimulation. But it is certain that the ionic concentration within the cell does not decrease during stimulation; in fact with prolonged stimulation it can be shown to increase, a change which in itself — supposing the ionic velocities unchanged — would produce a *positive* instead of a negative variation. To account for the negative variation it thus seems necessary to postulate a change in the ionic velocities of such a kind that the two become more nearly equal. This might conceivably be due to a decreased permeability to the cations, but such a supposition would contradict the fact that carbonic acid is freely lost during stimulation; the explanation most in conformity with the facts is that the general permeability undergoes increase so that all ions are free to diffuse from the cell; $\frac{u - v}{u + v}$ then diminishes, with corresponding decrease in the value of E. We reach the conclusion that the most probable explanation of the action current from a theoretical point of view is that the plasma membrane undergoes during stimulation a sudden increase in its permeability.

III. Experimental.

This brings us to the experimental part of our consideration. Are there other and independently determined facts indicating an increase in the permeability of the plasma membrane during stimulation? This question must be answered decidedly in the affirmative. In the first place, if a muscle is made to contract by immersion in a solution of a toxic substance like saponin, or if it is stimulated while under the influence of veratrin, or if it contracts in heat rigor or death rigor, the electrical change accompanying the contraction is *in the same direction as in normal stimulation :* it differs only in being irreversible; *i. e.*, the injured or poisoned parts of the tissue become permanently instead of transitorily negative relatively to the unaltered. At the same time the permeability is increased; the cells of dead tissues are invariably found much more permeable to dyes as well as to other dissolved substances (as shown in the loss of the normal osmotic properties) than during life. The normal electrical properties thus show a constant association with the normal semi-permeability of the plasma membrane; a permanently increased permeability, however induced, is associated with a marked and permanent decrease in the potential difference between the exterior and the interior of the cell; the most natural inference is that during stimulation a similar though temporary increase of permeability takes place.

Again, osmotic motor mechanisms exist in plants, where the movement depends on an increase in permeability relatively to the turgor-maintaining crystalloids of the irritable cells. The sensitive plant and the Venus' fly-trap are sufficient illustrations. In mimosa stimulation is associated with a loss of cell sap; water containing dissolved substances, undoubtedly those which maintain the turgor, leaves the pulvinus cells at such times (Pfeffer); *i. e.*, the permeability of the limiting membranes relatively to these substances is suddenly increased. Since the conditions of stimulation and its electrical manifestations are the same in these tissues as in muscle or nerve (as Burdon-Sanderson showed) we are warranted in pointing to this phenomenon as strong evidence in favor of our general proposition that stimulation is conditional on a sudden increase in permeability.

There is also evidence from the side of comparative physiology which points strongly in this direction. Favorable conditions for the examination of this question are met with in many lower organisms. The case of the Ctenophore swimming plate may be cited as an instance. As I described some years ago, these structures are stimulated to greatly increased activity in isotonic solutions of various neutral salts, particularly salts of alkali metals; during the rapid vibratile movements thus caused, the plate undergoes a progressive coagulation, accompanied by gradual swelling of its substance;[14] the entrance of water and the profound alteration in the structure of the coagulated plate — such that it falls to pieces on shaking — are sufficient evidence that a marked increase of permeability is associated with the strong stimulation caused by the solution. The stimulating as well as the coagulative and water-absorbing effects of (*e. g.*) an isotonic sodium chloride solution are prevented by the addition of a little magnesium and calcium chloride; in such a mixture the plates remain living and normal for many hours.[15] Strong stimulation is thus associated with marked increase of permeability in this tissue; the stimulating action of the pure solution and its permeability-increasing action are simultaneously checked by the alkali-earth salts. The same is true of its toxic action.

Another organism which gives remarkably unequivocal evidence of the truth of the present theory is the larva of the marine annelid Arenicola cristata. I have in an earlier paper described experiments with this organism bearing on the present problem;[16] further experiments carried out during the past two summers have confirmed and extended the results then reported. The larva is a free-swimming, strongly heliotropic trochophore about one third of a millimetre long; it has at this stage three body segments and a well-developed musculature of unstriated longitudinal fibres; and the tissues are remarkable for being permeated with a yellow or brownish water-soluble pigment. This is the peculiarity which fits the organism more especially for the purpose of the present investigation. The pigment during life is contained within the cells; at death or under the action of any cytolytic substance it diffuses into the water and colors the latter a distinct yellow. Its exit is to be regarded as evidence of marked increase of permeability. A precisely similar loss of pigment occurs during strong chemical stimulation, and the more rapidly the more intense the stimulation. I have described instances of this phenomenon in the paper cited; if the larvæ are collected in large masses in a watch glass — most readily by taking advantage of their strong positive heliotropism — and are then brought into pure isotonic sodium chloride solution, a strong and persistent muscular contraction to about half the normal length at once follows; the larvæ remain in a state of extreme contraction for fifteen seconds or so, and only gradually relax; during the period of contraction the diffusion of pigment into the solution is readily observed, particularly where the larvæ are massed, and the whole solution becomes colored a bright straw yellow; the organisms on closer examination are then found to be distinctly lighter colored than before. These characteristic effects are found in all solutions which stimulate intensely; if the stimulating action is checked, as by the addition of a little calcium or magnesium chloride to the sodium salt solution, the loss of pigment is also checked to a proportionate degree (see below, p. 212). The strong and irreversible stimulation produced by more concentrated solutions of lipoid solvents, like chloroform, ether, benzol, is also associated with rapid loss of pigment; weaker solutions of these substances produce normal reversible anæsthesia without loss of pigment. It should be noted that this difference in the action of lipoid solvents according to concentration is equally characteristic of vertebrate muscle, and probably of irritable tissues in general.

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[14] LILLIE: This journal, 1906, xvi, p. 117.

[15] LILLIE: *Ibid.*, 1908, xxi, p. 200.

[16] LILLIE: *Ibid.*, 1909, xxiv, p. 14.

If instead of pure isotonic ($m/2$) sodium chloride a mixture of 25 c.c. $m/2$ NaCl + 1 c.c. $m/2$ $CaCl_2$ is used, a totally different effect is seen. At the contact of the solution there is only a slight temporary contraction followed by immediate relaxation; there is no appreciable loss of pigment, and the larvæ exhibit for a few minutes a completely normal appearance and behavior, showing the usual vigorous swarming movements with strong positive phototaxis. After several minutes the solution begins to affect the organisms injuriously and the phototaxis disappears; later degenerative changes set in, accompanied by a gradual loss of pigment, indicating the progress of a slow cytolytic action; the larvæ nevertheless remain living in such a solution for many hours, while in the pure *m*/2 NaCl they rapidly die and disintegrate. Thus the calcium, in addition to exercising an *anti-stimulating*, exerts also an *anti-toxic* action; both effects are associated with a prevention of the rapid increase in permeability which the pure salt solution produces. The antitoxic action can of course be greatly increased by adding other salts; in appropriate mixtures of sodium, calcium, and magnesium chlorides the larvæ live and develop almost as in normal sea-water; the action of calcium chloride alone, while well marked, is incomplete. It should be noted that pure and calcium-containing sodium salt solutions show analogous differences in their action on vertebrate muscle, pure solutions inducing twitching and being more injurious to the tissue than the calcium-containing solutions in which the muscle remains quiet. Here also the twitching effect is to be regarded as an index of increased permeability; but both stimulating and permeability-increasing actions are much more intense in the embryonic invertebrate muscle of the above experiments.

Similar experiments were performed with pure and calcium-containing solutions of the following series of sodium salts in *m*/2 concentration: $NaCOOCH_3$, NaBr, $NaNO_3$, $NaClO_3$, NaI, NaCNS. The results with the first four salts were identical with those just described; with sodium iodide the antitoxic and anti-stimulating actions are less marked; with sulphocyanate the addition of calcium has a comparatively slight effect. The intensity of the stimulating action is greater with the salts toward the end than toward the beginning of the series, and the counteracting action of the calcium is less. A relation between stimulating action and colloid action is thus again seen.[17]

Lithium salts show similar relations, but lithium chloride stimulates less powerfully than sodium chloride, and correspondingly induces a less rapid loss of pigment. Both effects are checked by calcium chloride, and in the mixed solution the larvæ exhibit a normal appearance and behavior for some minutes. Lithium thus resembles sodium in its action on the neuromuscular system of Arenicola larvæ as well as of vertebrata.

On the other hand, the stimulating and toxic action of cæsium chloride is intensified by adding calcium chloride to the solution. Pure $m/2$ CsCl produces well-marked contraction and loss of pigment, followed in several seconds by relaxation; the calcium-containing solution causes an intense and much more persistent contraction accompanied by a decidedly more marked and rapid loss of pigment. The larvæ also die sooner in the mixed than in the pure solution. Thus increase in the permeability-increasing action is associated with increase both of the stimulating and of the toxic action.

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[17] *Cf.* my former experiments: this journal, 1909, xxiv, p. 459.

Pure isotonic solutions of potassium salts (KCl, KBr, KNO_3, KI, KCNS) also stimulate strongly and cause exit of pigment as above, but the action is not appreciably influenced in either direction by the addition of calcium chloride. Magnesium chloride, on the other hand, checks, and if present in sufficient proportion entirely prevents, both effects. This is illustrated by the following series. Larvæ were brought in the usual manner into the following solutions:

1. 80 volumes $m/2$ KCl + 20 vols. $m/2$ $MgCl_2$
2. 70 " " + 30 " "
3. 60 " " + 30 " "
4. 50 " " + 50 " "
5. 30 " " + 60 " "
6. Pure $m/2$ $MgCl_2$

In the pure magnesium chloride solution there is neither loss of pigment nor muscular contraction, the larvæ remaining rigid, extended, and motionless (except for the cilia which remain active) in a state of complete muscular anæsthesia. In mixtures with a high proportion of magnesium (solution 5) essentially the same effects are seen. With further increase in the proportion of potassium a stimulating action makes its appearance; this is slight in Solution 4 and accompanied by a slight loss of pigment; the degree both of the contraction and of the loss of pigment (and also of an associated agglutinating action) increases steadily with further increase of potassium, though both are distinctly less marked in Solution 1 than in pure $m/2$ KCl. Thus the two externally quite different effects of the solutions, increase of permeability and stimulation, vary in a closely parallel manner.

Other similar instances could be given, but the above are sufficient to illustrate the general nature of the observations. In general a cytolytic or permeability-increasing action seems equivalent to a stimulating action, provided the change is sufficiently rapid. It is to be expected that a tissue with a rapid time coefficient of stimulation like nerve or voluntary muscle will in general show less responsiveness to a cytolytic action, unless the latter is unusually rapid, than a more gradual tissue. During the past two years I have investigated in some detail the stimulating or contraction-producing action of various cytolytic substances on frogs' voluntary muscle; most of these produce slow steady contraction culminating in rigor; but it is possible by certain forms of artificial sensitization so to modify the irritability of the muscle that solutions of typical cytolytic substances, like saponin, chloroform, soap, bile salts, foreign blood sera, etc., produce on their first contact with the tissue quick vigorous contractions closely resembling the normal. This is done by previously immersing the muscle for several minutes in a bath of a pure isotonic solution of a neutral sodium salt; the most effective for this purpose are iodide and sulphocyanate, though chlorate, nitrate, bromide, and even chloride show a similar action, to a degree diminishing in this general order. Muscles thus treated and then instantly immersed in a solution of a cytolytic substance in physiological salt solution show in many cases — instead of the usual slow, steady shortening — contractions which are quick, vigorous, and often accompanied by active twitching. The contractions thus produced — *e. g.*, with saponin or digitalin — not only are more energetic and approach more closely to the normal than those similarly produced in unsensitized muscle, but they pass over more quickly into a condition of rigor; and this rigor with the associated coagulation is in many cases distinctly more pronounced than in the control unsensitized muscle of the same animal.[18] I have obtained these effects in varying degree with all of the following substances: chloroform, saponin, solanin, digitalin, agaricin, aconitin, bile salts, sodium oleate, foreign blood sera (horse, dog), tetanus toxin, rattlesnake venom. The stimulating action shows a general parallelism with the cytolytic (hæmolytic) action; this in its turn is the result of marked and irreversible increase of permeability. Substances whose specific action is to increase the permeability of the plasma membranes thus show as a class definite stimulating action.

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[18] These experiments are partly described in the Proceedings of the Society for Experimental Biology and Medicine, New York, 1910, vii, p. 170.

IV. CONCLUSIONS.

The evidence that a rapid increase in surface permeability is the critical change in stimulation is thus derived from many sides. This view clearly implies that the electrical current acts directly or indirectly by altering permeability. As already seen, Nernst's theory refers the action of the current to an increase in the concentration of ions at the membrane, a polarization being thus produced which must have a certain minimal value for stimulation. If we accept this theory, we must also assume that an intimate connection exists between the state of polarization of the plasma membrane and its permeability. Now, on the present theory, any increase in the ionic permeability of the membrane does produce a corresponding change in its polarization, but the change is in the nature of a decrease of an already existing polarization — *i. e.*, is a *depolarization*, which evidently may vary in degree. This depolarization, since it invariably accompanies stimulation, must be considered an essential feature of the process; there are indeed reasons for believing that the depolarization, and not the mere increase of permeability as such, is the really critical change in stimulation. The facts of electrical stimulation favor this view. The electrical current, when passed through an irritable tissue, stimulates just where its polarizing effect, if produced in the manner conceived by Nernst, is opposed in direction to the already existing or "physiological" polarization, *i. e.*, at the side adjoining the cathode; the current thus appears to stimulate only where its effect is to *depolarize*, — *i. e.*, to counteract to a greater or less degree an already existing polarization.[19] From this point of view electrical and other forms of stimulation may be regarded as essentially similar in their mode of action. The current stimulates, not by producing a condition of polarization at previously unpolarized membranes, but by decreasing or nullifying one already existing. At the anodal side where the preëxistent physiological polarization is not compensated but reinforced by the external current there is no stimulation when the current is made, but a well-marked one when it is broken — again when the polarization is suddenly diminished.

But is a depolarization, during which the permeability of the membrane remains unaltered, sufficient for stimulation? It is doubtful if simple depolarization, unaccompanied by increase of permeability, is possible for any irritable tissue; the available facts indicate that the plasma membrane is so constituted that its normal semi-permeability during rest *depends* on its being electrically polarized, so that depolarization necessarily involves a simultaneous increase in permeability; hence an external current in stimulating not only depolarizes but by that very action increases the permeability of the plasma membrane. All the facts of stimulation indicate that the semi-permeability of the membrane in an irritable tissue is a matter of very delicate adjustment, such that any decided chemical or physical change in the membrane alters its permeability, and usually in the direction of an increase;[20] hence a great variety of such changes may act as stimuli. Just why the condition of polarization of the membrane should determine its permeability is difficult to say at present; it is clear that the state of the colloids must be influenced by the distribution of ions; one might also suggest that the double electric layer with the interposed thin surface film is comparable to a condenser; in any such system the material between the two layers is in a state of compression due to the attraction of the opposite charges. This effect, however, must be slight unless the potential difference under static conditions is much greater than that usually assumed; but it seems possible that the density and hence the permeability of the membrane may thus be influenced. Girard has found that an electrically polarized membrane of pig's bladder has a different permeability from the same membrane in the unpolarized state, and also that not merely the degree but the orientation of the polarization is a factor in determining the permeability; a polarized membrane may thus be unequally permeable in the two directions.[21] The case of the plasma membrane appears to be analogous. At all events it is clear that the permeability increases during electrical stimulation; this is proved by the case of the osmotic motile mechanisms of plants, above cited, in which the conditions of electrical stimulation are the same as those of muscle and nerve, and where the very condition for the movement is an increase in the permeability of the limiting semi-permeable membranes.

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[19] *Cf.* BRÜNINGS: Archiv für die gesammte Physiologie, 1903, c, p. 367.

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[20] It should be remembered that *irritable* cells and tissues are under discussion here. Undoubtedly the plasma membranes of many cells are much more resistant to changes in their permeability; *e. g.*, immature egg-cells.

[21] Girard: Journal de physiologie et pathologie générale, 1910, xii, p. 471.

The assumption of a reciprocal interdependence between the polarization of the membrane and its permeability throws light on another characteristic feature of the stimulation process, — the fact, namely, that the effect of a local stimulus is never confined to the region directly stimulated but spreads to adjacent regions. In other words, the region already in a state of excitation excites the adjoining regions, so that an excitation wave travels from point to point along the irritable element at a more or less rapid rate. On the present theory the wave of excitation is accompanied by, or equivalent to, a wave of depolarization, *i. e.*, of increased permeability. It is evident that if the increase of permeability were confined to the point of stimulation there could be no excitation of the irritable element as a whole, but, as we have seen, the region where permeability is locally increased by any means undergoes depolarization, and it is to be assumed that this region, acting then as cathode, influences electrically the adjoining regions, depolarizing these in turn and causing a corresponding increase in permeability; in this manner the effect is propagated along the membrane [22] at a rate which in any particular case will evidently depend on the sensitivity or quickness of response of the tissue — *i. e.*, in general on the duration of the latent period of stimulation: when this is very short, as in nerve, the propagation velocity will be high.

The transmission of the excitation state, on the present theory, is a direct consequence of the above interdependence between depolarization and increase of permeability. If a localized depolarization produced no effect on the membrane itself, it is clear that its transmission with virtually unaltered intensity for an apparently unlimited distance, as in nerve, would be inconceivable. But a depolarization which at the same time involves an increase of permeability — *i. e.*, furnishes the condition for further depolarization — is theoretically transmissible to an indefinite distance along a membrane polarized in the above manner. It thus seems probable that the condition called irritability depends on the existence of a close interdependence between the physiological polarization of the limiting membrane and its permeability, depolarization corresponding to or inducing increased permeability; hence a local depolarization involves a local increase in permeability which is propagated along the membrane. The region already in a state of excitation affects the adjoining regions in a manner essentially identical with that in which the excited region of one muscle may stimulate the nerve of another. Tissues in which the permeability of the plasma membrane is very quickly and sensitively affected by changes in the potential difference across the membrane, would show high irritability and rapid conduction of the depolarization or excitation wave. This condition has apparently reached its highest development in the nerve fibres of warm-blooded vertebrata.

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[22] The best inorganic parallel to this process is the progressive dissolution of the film of peroxidate covering at intervals the surface of the mercury in the rhythmical catalysis investigated by Bredig and his collaborators. *Cf.* Antropoff: Zeitschrift für physikalische Chemie, 1908, lxii, p. 513. *Cf.* my earlier paper on the present subject: This journal, 1909, xxiv, p. 18.

In electrical stimulation the essential conditions appear to be that the potential difference across the membrane is decreased to a certain critical degree, and at a certain critical rate, by the opposed electromotive force of the external current. The precise value of this decrease, or depolarization, as measured in volts, has not been determined; but in the case of a highly irritable tissue it must be very slight — probably much less than that of the action current of the succeeding response. Upon this initial depolarization follows, as we have seen, a local increase in the ionic permeability of the membrane, producing at the point stimulated the characteristic electromotor response of the tissue itself; this change affects the adjoining regions of the membrane, in the same manner as the original external current,— and so the impulse spreads. For the indefinite propagation of such an impulse it is evident that the depolarizing effect of the action current at the adjacent regions of the membrane must be sufficient to induce there an increase of permeability equal to or greater than that at the original point of stimulation, — otherwise the impulse will die out. The rate of its propagation will naturally depend on the various specific peculiarities of the membrane.

It is clear that a necessary condition for rapid propagation of the state of excitation is a *short latent period of stimulation*, since the stimulation of each successive region in a stretch of nerve (*e. g.*) is due to the change in the adjoining region. There should thus be a general inverse proportionality between the duration of the latent period and the velocity of conduction. That this proportionality holds qualitatively is beyond a doubt, but the quantitative data at hand are insufficient for a precise and detailed examination of the conditions.

The latent period of excitation, strictly speaking, is the time interval between the exciting change (considered as momentary) and the beginning of the electrical response at the point excited. There are as yet no accurate measurements of this interval. But what is really required to test the present hypothesis is some objective measure of the rate at which the electrical change develops — *i. e.*, at which the membrane undergoes depolarization. Such an estimate can be obtained from the duration of the rising phase of the action current curve. Measurements made by Garten with the thread galvanometer give for this interval in the frog's sciatic nerve a duration of *ca.* 0.001 second at 18°; at 32° the duration was *ca.* 0.00055 second;[23] rise of temperature thus increases the velocity of this change in virtually the same proportion as the propagation velocity of the nerve impulse, indicating a dependence of the two changes on the same condition.[24] This condition, on the present theory, is increase in the ionic permeability of the membrane; and since the temperature coefficient is that of chemical reactions, it may be inferred that the permeability change is due to a chemical alteration of the membrane — probably a change in the aggregation state of the colloids.[25] Garten finds a correspondingly slow rise (*ca.* 0.07 second) in the slowly conducting nonmedullated (splenic) nerves of mammals. Babkin[26] has compared the time relations of the electrical variation in a slowly contracting (hyoglossus) and a rapidly contracting (sartorius) muscle of the frog at different temperatures; the former shows a slower rise of the action current curve — *ca.* 0.006 second for sartorius and *ca.* 0.009 second for hyoglossus — and a correspondingly slower conduction rate. The latter thus appears directly dependent on the rate of the electrical change. The rate of this change, however, is, on the present theory, an index of the rate of the permeability change. This last is to be regarded as a measure of the responsiveness of the tissue and hence as determining the rate of conduction.

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[23] *Cf.* GARTEN: "Die Produktion von Elektrizität," in Handbuch der vergleichenden Physiologie, edited by Winterstein, Jena, 1910, iii, pp. 135 *seq.*

[24] *Cf.* K. LUCAS: "On the relation between the electric disturbance in muscle and the propagation of the excited state," Journal of physiology, 1909, xxxix, p. 207.

[25] The temperature coefficients of colloid changes are insufficiently known. For some changes, such as the swelling of gelatine in water, they are high, and it is assumed above that colloid reactions in general probably have the usual temperature coefficient of chemical reactions. The fact that vital processes so generally show the chemical temperature coefficient seems inconsistent with any other assumption.

[26] BABKIN: Archiv für die gesammte Physiologie, 1908, cxxv, p. 595.

Some further evidence that stimulation involves changes in the electrical polarization of the limiting membranes of the irritable tissue may now be briefly reviewed. It has long been known that artificial semi-permeable precipitation membranes become the seat of a polarization when a current is passed, and that this polarization may serve as a secondary source of current.[27] Lapicque has shown that the *degree* of the polarization thus produced at artificial membranes, as measured by the voltage of the secondary or polarization current, is related to the *duration* of the polarizing current in a manner quantitatively identical with that in which the stimulating effect of a current is related to its duration.[28] This result might have been predicted from Nernst's theory, and constitutes a further and striking proof of the connection between stimulation and polarization at the membranes.

But if the electrical current excites by altering the polarization at semi-permeable surfaces, it is clear that chemical or mechanical excitation, which produces effects indistinguishable from electrical excitation, must produce the same alteration. Now these agencies cannot originate a polarization at such surfaces; they may however alter an existing state of polarization by altering the character of the membrane which is the seat of the polarization. This consideration explains why such diverse agencies act alike. There is definite evidence that the current does not act simply by polarizing the membranes, but rather by partially compensating, and so decreasing, a preëxistent polarization (inference from Pflüger's law). Other forms of stimulation produce the same effect by temporarily increasing, through some chemical or physical alteration, the ionic permeability of the membrane. From this point of view it becomes clear why the various stimuli have an identical action on the irritable tissue; their common effect is depolarization, with which is associated an increased permeability.

Confirmatory evidence of the truth of this general point of view is afforded by the characteristic loss or lowering of irritability during excitation. If the depolarization of stimulation is associated with a free permeability of the plasma membrane to ions, the electrical current should be relatively or absolutely without action on irritable tissues at the height of stimulation. That this is in fact the case is shown by the existence of the so-called refractory period; this period varies in duration for different tissues, being short for quickly responsive tissues like voluntary muscle and nerve (*ca.* 0.003 second for frogs' muscle,[29] 0.001 for nerve[30] at 14°–15°), and longer for slower tissues like the vertebrate heart (1 second or more); the fact that contractions are normally maximal in this tissue is probably directly connected with the existence of an unusually distinct and prolonged refractory period. What is significant from the present point of view is the fact that in all of these instances *the refractory period has approximately the same duration as the electrical variation*,[31] *i. e.*, as the period of increased ionic permeability. Its duration is also affected by temperature in the same manner.[32] It is evident that these relations, established quite empirically, receive a consistent theoretical explanation on the present theory. During the period of increased permeability the membrane offers little or no resistance to the passage of ions; hence it is no longer the seat of a polarization, and the polarizing or depolarizing action of the current should no longer be possible. To this theoretical requirement corresponds the observed fact of a lowered or abolished irritability immediately after stimulation.

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[27] *Cf.* Overbeck: Annalen der Physik, 1891, N. F. xlii, p. 193; Springmann: *Ibid.*, 1894, li, p. 140; Ostwald: *Loc. cit.*

[28] Lapicque: Comptes rendus de la Société de Biologie, 1907, lxiii, p. 37.

I have not attempted in the present paper to consider the possible nature of the connection between altered polarization or altered permeability of the plasma membrane, and altered oxidation and energy production by the protoplasm, but merely to summarize and coördinate the facts and theoretical considerations which point to the plasma membrane as the seat of stimulation and to an increase in its permeability as the essential change involved. The reference of the one constant external manifestation, the action current, to a decrease in a preëxistent polarization following increased ionic permeability, is largely a theoretical deduction from the phenomena of concentration cells; the unusual feature of this explanation, from a physico-chemical point of view, is that it postulates the existence of membranes whose permeability readily undergoes rapid and reversible changes under the influence of, or in correlation with, changes in their electrical polarization. Proofs that the permeability of living membranes may change reversibly and in either direction are not wanting;[33] there is also indication that the permeability of membranes other than those of living cells may be altered by electrical polarization. The present theory of stimulation requires that such reversible changes in permeability should in highly irritable tissues occur with great quickness and readiness; thus Garten's measurements indicate that the duration of such a change may in nerve be less than .001 second. Artificial membranes having such properties are as yet unknown; the belief that the plasma membranes of muscle and nerve are thus characterized is as yet mainly a deduction from the theory; but if the electrical variation is due to increase of ionic permeability, such a deduction seems unavoidable.

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[29] *Cf.* Bazett: Journal of physiology, 1908, xxxvi, p. 414.

[30] Boycott: *Ibid.*, 1899, xxiv, p. 144.

[31] *Cf.* Tait: Quarterly journal of experimental physiology, 1910, iii, p. 221.

[32] *Cf.* Snyder: This journal, 1908, xvii, p. 384.

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[33] *Cf.* HÖBER: *Loc. cit.*, chapter 10; egg cells immediately after normal or parthenogenetic fertilization furnish good illustrations. *Cf.* my recent paper, this journal, 1911, xxvii, p. 289, especially pp. 300 *et seq.*

EFFECT OF INCREASED TEMPERATURE OF THE CAROTID BLOOD.

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IN the course of a series of experiments on the effect of increase of temperature on the heart and its nervous mechanism, we were at first inclined to accept the results of Kahn with respect to heart rate, published in his paper "Über die Erwärmung des carotiden Blutes." [2] Certain of our results led us to investigate more fully his methods and findings. We perceived that his method of procedure was open to certain objections and have repeated some of his experiments with additional precautions. We have demonstrated that in Kahn's experiments regarding the effect of warming the carotid blood certain factors were disregarded which have a very important bearing on the subject.

Briefly, Kahn's method was to enclose the carotid arteries in heaters, through which a stream of hot water was flowing, while registering temperatures in the gullet and rectum, and observing heart beat and blood pressure by means of a Cowl-Gad apparatus. By trephining and inserting a thermometer into the brain and comparing its readings with one in the gullet; we have found that the readings differ considerably. The following abstract of a protocol serves to illustrate this point.

Experiment 6. — Feb. 14, 1911.

Time.	Brain temperature.	Mouth temperature.
2.28	35.5	34.8
3.18	36.0	34.8
3.30	38.5	38.2
3.35	40.5	39.0
4.11	35.2	34.2
4.27	38.0	36.4
4.34	39.4	38.2

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[1] The experiments reported in this paper were performed during the author's tenure of the George Brown Memorial Scholarship.

[2] KAHN: Archiv für Physiologie, 1904, p. 8.

It may be readily understood that the temperature in the rectum does not give sufficiently definite information, since variations in the heart temperature might take place within comparatively wide limits before the rectal temperature showed any sign. This variation in heart temperature must have been present in Kahn's experiments, since no provision was made for cooling the return flow from the brain, and the overflow of heated blood from the superior vena cava into the right auricle must have exerted its effect locally on the heart as well as on the cardiac reflex described by Mansfeldt.[3] The effect of increased temperature on the perfused mammalian heart is known to result in acceleration. The following section of a protocol illustrates this direct local effect due to heated blood when both accelerators and vagi are cut.

Experiment 10.—Dog, 18 kilos. March 2, 1911.

Time.	Heart temperature.	Heart rate.
4.36.00	36.2	144
.10	40.1	156
.30	41.2	162
.40	39.2	162
4.37.00	40.0	174
.30	42.0	186
4.38.10	38.0	174

Our procedure, therefore, was as follows: Dogs were used in all the experiments. They were anæsthetized lightly with urethane, sometimes morphine in doses of 2.5–5 mgm. per kilo body weight, and an ether-chloroform mixture. A median skin incision was made from the level of the angle of the jaw to the episternal notch and the structures exposed. The sterno-mastoid, sterno-hyoid, and sterno-thyroid muscles were ligatured, cut, and retracted. The carotid artery was cleaned from the fascia and the vagus nerve for about five inches. The trachea was divided, a cannula was tied in, and the freed end retracted over the sternum. The vagi were enclosed in thin rubber sheets moistened with saline, and a flat plate cooler was inserted between arteries and nerves so as to insure the nerves from damage by the heat. The carotids were then enclosed in the heaters of the type described in Kahn's paper. The vertebral arteries of both sides were tied. The right external jugular vein was cleaned and enclosed in a cooler of the same type as the heaters, but longer and of larger bore. The left external jugular was used for inserting the thermometer into the right auricle. The internal jugulars were tied. The frontal sinus was opened and a trephine hole, large enough to admit the brain thermometer, was made in the posterior wall. The thermometer tip rested in the temporal lobe. Blood pressure was taken from the femoral artery and recorded by a von Recklinghausen tonometer. An injection cannula was inserted into the femoral vein of the other side. When respiratory variations were studied, the animal was made to breathe into a glass jar of 10 litres' capacity, and the recorder which shows volume was of the type described by Jerusalem and Starling.[4] In studying vaso-motor changes the forefoot of the dog was enclosed in a plethysmograph, and an air-tight piston recorder of new type, to be described elsewhere, was used.

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[3] Mansfeldt: Archiv für die gesammte Physiologie, 1910, cxxxiv, p. 598.

Kahn carried out his experiments upon unanæsthetized animals, a procedure to which objection must be made. The sensory stimuli which are engendered by operating on and applying apparatus to such animals must have a direct and indirect effect on the heart action. The first effect of such stimuli would be to bring on pain dyspnœa, and the carbon dioxide content of the blood would be altered to such an extent as to preclude the possibility of making correct deductions from the heart tracings (Henderson).[5] It is best here to quote a protocol of one of Kahn's experiments (kind of animal not mentioned):

Time.	Temperature in gullet.	Temperature in rectum.	Blood pressure.	Heart rate.
4.36	37.2	38.0	85	47
4.41	38.1	38.0	90	50
4.50	38.8	38.2	80	52
4.57	40.2	38.6	100	52
5.70	41.6	39.0	100	48
5.14	42.0	39.2	100	51
5.20	42.1	39.2	100	56
5.27	39.3	39.1	89	52
5.36	37.8	38.5	85	48

[4] Jerusalem and Starling: Journal of physiology, 1910, xl, 279.

[5] Henderson: This journal, 1910, xxvii, 152.

As is seen by the protocol, the heart rate of the animal at the outset was 282 per minute. Surely with such a rapid rate an increase in any way proportional to temperature could not be expected. In fact many of the animals experimented on must have been in the condition known as acapnia from the commencement. To obviate difficulties of this type our animals were lightly anæsthetized, in fact just enough anæsthetic was used to prevent the animal from going into the condition of acapnia without rendering the centres insensible to the changes of temperature. With this end in view the animál was made to breathe through a large-bore rubber tube three feet long, thus increasing the dead space. In some of the experiments the respirations were recorded, and in others, where it was thought necessary to have little or no variation of carbon dioxide, curara was given and artificial respiration used.

A. **Respiratory changes.** —

Protocol (*a*). *Experiment X.* Dog, 27 kilos. Morphine, urethane, ether.

Time.	Brain temp.	Resp. rate.	Resp. vol. c.c.
2.15	36.2	18	400
2.45	37.0	24	410
3.15	35.0	24	390
4.05	36.0	26	375
4.15	37.0	27	375
4.20	40.0	78	250

Protocol (*b*). — *Experiment 2.* Dog, 19 kilos. Morphine, urethane, ether.

Time.	Brain temp.	Resp. rate.	Resp. vol. c.c.
12.55	37.6	12	400
1.02	37.9	18	275
1.30	37.8	20	225
1.40	38.2	24	200
1.47	38.5	42	175
2.00	39.1	30	190
2.29	39.6	18	350
3.00	37.8	12	475
3.25	37.0	12	425

Protocol (*c*). — *Experiment 3*. Dog, 19.95 kilos. Morphine, 2.5 mgm. per kilo. Urethane, 4 gm.

Time.	Heart temp.	Brain temp.	Resp. rate.	Resp. vol. c.c.
...	36.2	36.8	18	150
12.24	36.0	38.0	12	325
12.25	36.0	38.0	12	290
12.50	...	...	12	350
1.05	36.4	38.5	12	425
1.23	36.5	39.0	12	450
1.45	36.5	39.0	12	475
2.25	36.3	37.7	...	...
3.35	36.0	36.9	10	475

In considering the effect of heat on the respiratory centre several factors must be recognized. Dogs depend for their heat loss mainly on the evaporation of water from lungs and air passages, and respond to external heat by an increased ventilation, as is observed by Scigliano[6] (guinea pigs) and Wood and Cerna[7] (dogs). On teleological grounds one would expect increased ventilation to result from central heating also, but the particular type of respiratory change is problematical. Kahn states that acceleration and shallowing of breathing were the effects noted in his experiments when the carotid blood was warmed, and for high temperatures he noted slowing and deepening. The part which sensory stimulation might play in causing these respiratory variations must not be lost sight of, and that Kahn recognized this is evident from his precautions in carefully insulating his heaters from the surrounding tissue. Insulation of the heaters with cotton wool such as Kahn practised could not prevent, we found, the escape of heat such as might affect the vagi. Scott[8] observed that when the vagi are cut increase in carbon dioxide causes a deepening of respiration but no increase in rate, in fact sometimes slowing. It seemed possible that the slowing observed by Kahn for high temperatures (42°) was due to a destruction of the vagi by heat escape from the heaters. This we completely avoided in our experiments by the use of the plate cooler. Other sources of sensory stimulation were also present in his experiments, — *viz.*, the effect of the heated blood from the carotids going to the sensory areas of the face and head, and the return flow of heated blood by the jugulars. In our experiments the latter error has been overcome by using coolers on the jugular.

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[6] SCIGLIANO: Archivio di fisiologia, 1911, ix, 297.

[7] WOOD and CERNA: Journal of physiology, 1892, xiii, 870.

[8] SCOTT: *Ibid.*, 1908, xxxvii, 301.

The former source of stimulation may be counteracted to a certain extent by anæsthesia, but the impossibility of entirely eliminating sensory reflex effects by this course is quite apparent to us. In protocol (*a*) it will be noted that an increase of brain temperature produces acceleration and shallowing of respiration. In protocols (*b*) and (*c*) a slowing of rate and increase of volume are seen to occur. Another type of response is the increase of volume without change in rate (protocol *c*). These differences may depend on slight variations in the depth of anæsthesia of the sensory side of the central respiratory mechanism, and would therefore be analogous to the varying effect of carbon dioxide inhalation with intact and cut vagi, as noted by Scott.[8] These are points which demand further investigation.

B. **Vaso-motor changes.** — Recording the volume of a forefoot with a plethysmograph, the vaso-dilatation was observed to go hand in hand with the temperature changes. As seen in the protocols, the dilatation of peripheral vessels conforms remarkably well to temperature increases. This dilatation was observed in slow-heating experiments to take place considerably before the heart rate was affected by the same increase of temperature and before the respiratory changes. The vaso-motor centre appears to be more easily excited than the respiratory or heart centres. The reflex effect from the skin of the head is a possibility which we have taken into consideration. Observations on dogs in which arm volume was recorded and heat applied to skin areas showed us a slight vaso-dilatation. With human subjects we have observed the reflex vaso-dilatation to be more marked.

Experiment 3 (*a*). — Dog, 19.95 kilos. Morphine, urethane, ether.

Time.	Brain temp.	B. P.[9]	Pleth.[9]
12.00	36.8	136	1
12.24	38.0	160	48
12.25	38.0	160	50
1.05	38.5	144	82
1.23	39.0	144	96
1.45	39.0	148	138
2.25	37.7	152	158
3.25	36.9	140	74

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[9] B. P., blood pressure in mm. Hg. Plethysmograph variations are given in millimetres of rise of the recording lever.

Experiment 2 (b). Dog, 19 kilos. Morphine, urethane, ether.

Time.	Brain temp.	B. P.	Pleth.
12.30	38.2	128	0
...	...	124	10
12.55	37.6	192	6
1.02	37.9	152	23
1.20	37.7		38
1.30	37.8	124	42
1.40	38.2	132	43
1.47	38.5	156	45
2.00	39.1	140	53
2.29	39.6	152	65
3.25	37.0	132	25
3.30	36.9	132	25

C. **Changes in cardiac rate.** — The possibilities of error in Kahn's experiments regarding heart rate have been mentioned. Regarding the effect of the return flow from the head influencing the heart rate both locally and reflexly, it is not necessary to say anything further. Another factor in causing acceleration under conditions of such experiments must be discussed here. In the experiments in which the respiratory changes were recorded, we observed an increase of respiratory ventilation for increase of brain temperature, as has been pointed out. This increased ventilation might readily be sufficient to lower the carbon dioxide tension in the blood and thus cause changes in the rate of the heart itself and in the activity of the centres (Henderson).[5] The effect of increasing the percentage of CO_2 in perfusion fluid going to an isolated heart was observed by Jerusalem and Starling to be a slowing of the heart beat; conversely, on decreasing the CO_2 percentage, an acceleration. Experiments were also carried out by varying the percentage of CO_2 in the inspired air with similar results. These errors have been avoided, as pointed out above, by increasing the dead space or injecting curara. Our results obtained under these conditions are seen in the following protocols:

Protocol A. — Experiment 1. Dog, 16.3 kilos. Chloroform, ether, curara.

Time.	Heart temp.	Brain temp.	Heart rate.
2.20	37.0	38.2	156
2.21	...	38.3	168
...	...	38.4	180

Protocol A.—Experiment 1 (continued).

Time	Heart temp.	Brain temp.	Heart rate.
2.23	...	38.6	180
2.25	36.6	38.8	180
2.26	...	38.9	186
2.27	36.8	39.0	186
2.34	37.2	39.6	192
2.40	37.6	40.0	198

Protocol B.—Experiment 6. Dog, 10.9 kilos. Urethane, ether.

12.45	34.8	35.0	84
12.48	35.0	36.0	180
1.18	36.4	38.4	186
1.30	37.0	39.0	201

Protocol C.—Experiment 9. Urethane, ether, curara.

3.27	37	37.0	84
3.41	37	38.0	108
	37	39.2	144
3.42	37	39.3	150

Protocol D.—Experiment 15. Dog, 8 kilos. Morphine, ether.

12.00	36.3	36.0	102
12.02	36.3	37.0	117
12.04	36.6	38.1	117
12.08	36.6	39.0	126
12.09	36.6	39.0	135

Protocol E.—Experiment 5. Dog, 11 kilos. Morphine, urethane.

1.10	32.0	32.0	144
1.20	31.8	32.4	144
1.30	32.0	33.4	120
1.35	32.0	34.0	108
1.45	32.0	34.2	132
2.10	33.5	34.5	156
2.30	34.0	35.5	168
2.50	34.4	36.1	168
3.25	35.0	37.2	174

In nearly every case the effect of heating the brain was to produce heart acceleration. Protocols A, B, C, and D are examples of this.

The rate at which the changes of temperature were brought about had no influence on the heart acceleration in our experiments. Protocol E is an example of a preliminary slowing for increase of temperature followed by acceleration. This was observed in a few of our experiments.

Effect of heat on vagus centre. — In protocol F it will be seen that section of the vagi does not prevent the increase of heart rate for higher temperatures. In protocols H and I it will be seen that section of the vagi after the accelerators have been cut causes a rise in heart rate. It is evident that when the vagi only are cut, the impulses generated in the centres by the action of the heated carotid blood discharge on the heart by the accelerator mechanism. It is interesting to note in protocol I that after cutting the accelerators and cooling to 34.5 warming of the centres caused a preliminary drop in rate which could only be due to an increase in vagus tone and is probably analogous to the initial drop in rate in protocol E mentioned above. It is evident that heat to the centre may increase the activity of the vagus centre.

Protocol F. — Experiment 4. Dog, 17 kilos. Morphine, urethane, ether.

Time.	Heart temp.	Brain temp.	Heart rate.
2.45	35.0	35.2	168
2.53	35.5	36.0	174
2.55		36.5	180
3.00	35.6	37.3	186
3.10	36.0	38.0	186
3.20	36.5	38.6	192
	ONE VAGUS CUT.		
3.35	36.4	37.	174
	SECOND VAGUS CUT.		
3.47	36.0	36.2	168
4.00	35.5	36.9	180
4.03	35.7	37.5	192
4.20	36.4	39.0	198
4.33	36.6	39.5	204
4.40	36.4	38.5	198

Effect of heat on accelerator centre. — In the protocols dealing with this effect, G, H, I, it will be noted that there is a very general drop in rate following such section, if undertaken when the brain temperature is above normal, and any increase in rate subsequently can be traced to the direct effect of changes in heart temperature. These observations show clearly that the effect of heat on the centres is to cause an acceleration, and that this acceleration is accomplished mainly by the accelerator mechanism.

Protocol G. — Experiment 8. Dog, 20 kilos. Urethane, ether-chloroform, curara.

Time.	Heart temp.	Brain temp.	Heart rate.
12.20	36.0	36.5	150
12.25	36.0	37.6	156
1.12	35.0	36.0	180
1.29	35.5	37.0	204
2.23	35.6	36.5	174
2.28	36.0	37.0	192
2.43	37.0	38.0	228
2.46	37.0	38.5	228
2.48	37.2	39.0	228
2.49	ACCELERATORS CUT		
2.51	37.2	39.0	204
3.01	37.2	38.5	174
3.16	36.5	37.4	144
3.27	36.0	36.8	138
3.32	36.0	37.0	138
3.34	36.0	37.5	144

Protocol H. — Experiment 10. Dog, 18 kilos. Urethane, ether-chloroform, curara.

1.25	36.4	36.8	120
1.52	36.3	37.5	132
2.00	36.4	38.0	156
2.08	36.5	38.0	150
2.30	36.5	37.0	146
2.55	36.4	36.8	141
2.58	36.5	37.5	150
2.59	36.6	37.9	165
3.06	37.0	39.0	174
3.10	36.8	39.4	168
. . .	. . .	39.6	177
3.18	36.8	39.5	174

Protocol H.—Experiment 10 (continued).

ACCELERATORS CUT

Time.	Heart temp.	Brain temp.	Heart rate.
3.19	...	39.3	159
3.25	36.8	39.0	150
3.40	36.0	36.5	147
4.00	36.0	37.5	147
4.09	36.0	38.4	147
4.14	36.0	39.0	141

VAGI CUT

4.15	36.0	39.0	156
4.20	36.0	39.0	156

Protocol I.—Experiment 12. Dog, 9.07 kilos. Urethane, ether.

12.10	36.8	38.0	174
12.12	36.8	39.0	190
12.30	35.0	35.1	156
12.32	35.0	35.4	156
12.34	35.2	37.0	162
12.37	35.6	38.0	165
12.44	35.6	39.0	192

ACCELERATORS CUT

12.45	35.6	39.0	171
12.47	35.8	39.0	154
12.48	35.8	39.3	147
1.11	34.5	34.5	132
1.17	34.5	36.0	118
1.20	34.5	37.0	118
1.25	34.6	38.0	124

VAGI CUT

1.30	34.5	37.0	132
1.33	34.3	36.0	120

The author wishes to thank Professor V. E. Henderson for his many and valuable suggestions and criticisms.

SUMMARY.

The results of warming the brain by heating the carotid blood:

I. An increase in heart rate which is due to increased accelerator tone.

II. In some cases a primary decrease in rate is noted due to an increase in vagus tone.

III. An increase of blood in the periphery. The vaso-motor response seems to be more delicate than that of heart or respiratory mechanisms.

IV. An increase in respiratory ventilation. This usually is due to an increase in rate with shallowing, but we have observed increase in depth with no decrease in rate and with decrease in rate. These differences are probably due to differences in activity of the sensory side of the respiratory mechanism.

THE EFFECT OF EXERCISE UPON THE VENOUS BLOOD PRESSURE.[1]

By D. R. HOOKER.

[From the Physiological Laboratory of the Johns Hopkins University.]

THAT exercise produces a rise of venous pressure is evident from even casual observation of men and animals doing muscular work; the superficial veins stand out conspicuously, and upon palpation they are found to be hard as well as full. The extent of this rise of pressure has not been investigated, nor have explanations been advanced to account for the condition.

Owing to the difficulties which beset attempts to investigate the subject of venous pressure in animal experimentation, we have tacitly accepted the view that the veins serve merely as wide channels where the blood in excess of that needed for the arterial circulation is collected, and through which, by gentle gradations of pressure, it is conveyed back to the heart in such a way that the feeding pressure of the latter organ is practically constant. There is always enough, and there is never too much, blood to supply this delicately regulated pump. The fact that normal muscular exertion causes a considerable rise of venous pressure is sufficient to indicate that that view requires modification. In a sense such a rise of venous pressure might be regarded as a transient alteration of circulatory conditions analogous to the results observed upon stimulation of sensory nerves, the intravenous infusion of salt solution, etc. This exercise rise of pressure may be maintained, however, for a considerable time, long enough certainly to permit of readjustment if the circulatory economy called for it. It would seem, therefore, since this elevation of pressure is maintained throughout the period of exercise, that it may serve a specific purpose and be quite in keeping with the proper function of the mechanism.

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[1] A preliminary report of this work is printed in the Proceedings of the American Physiological Society, HOOKER and WOLFSOHN: This journal, 1910, xxv, p. 24.

Most of the observations reported in this paper were made upon man. The instrument used is a modification of that used by von Recklinghausen,[2] and has already been described.[3] The principle upon which the method depends may be briefly stated. The pressure in any vein is the resultant of two factors, (*a*) the force of the heart beat remaining after the peripheral resistance has been overcome, and (*b*) the weight of the hydrostatic column extending from the vein to the heart level. If the vein observed lies below the level of the heart, the hydrostatic column adds itself to the pressure in the vein caused by the heart beat; conversely, if the vein lies above the heart level, the hydrostatic column becomes negative in value and acts to reduce the pressure otherwise produced. In man the pressure within a superficial vein may be measured by determining the force, expressed in centimetres of a column of water, necessary to overcome the pressure within the vein. Having, for example, determined the pressure necessary to cause a superficial vein of the hand to collapse while the hand is resting on the knee to be 20 cm. of water, a correction may be made by deducting the difference in level between the vein and the heart, about 12 cm., which gives the venous pressure when referred to the heart level as 8 cm. of water. The level of the heart must be arbitrarily chosen. In this work it was taken as the mid-point of the antero-posterior diameter of the body at the tip of the sternum. The instrumental error of the method is less than 2 cm.

In different individuals the venous pressure determined by this method may vary from 2 to 16 cm. The average value is about 9 cm. The variation to be observed in normal individuals is probably to be ascribed to uncontrolled experimental conditions, since the same individual may show similar variations under altered circumstances.

In the experiments on man here recorded, the subject was seated on a stationary bicycle such as is used in gymnasia for exercise. Both hands rested on the handle bars. With one hand the subject maintained his balance and position; the other was left free and relaxed as much as possible, and on it the determinations were made. No record was kept of the arterial pressure, the heart rate, or the respiratory rate. From the work of others,[4] it may be assumed that there was an increase of all these. Further no means were available to ascertain the amount of physical exertion made. In most of the subjects the exertion was, however, violent, as indicated by profuse perspiration, dyspnœa, and subsequent fatigue.

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[2] v. RECKLINGHAUSEN: Archiv für experimentelle Pathologie und Pharmacologie, 1906, lv, p. 463.

[3] HOOKER and EYSTER: The Johns Hopkins Hospital bulletin, 1908, xix, 274.

[4] BOWEN: This journal, 1904, xi, p. 60.

The protocols of the experiments on man follow:

Dec. 1, 1909. Subject, W. Exercise, stationary bicycle.

Time.	Venous pressure.	Remarks.
2.27	3.75	Control record.
2.28	....	Exercise begun.
2.30	1.75	....
2.35	0.75	....
2.40	3.75	....
2.45	3.75	....
2.50	5.75	....
2.55	7.75	....
3.10	10.75	....
3.12	10.75	Exercise stopped.

The exercise lasted forty-four minutes. The venous pressure rose 7 cm. During the first of the exercise the respiration was accelerated. This subject served also for the experiment of Dec. 13, 1909.

Dec. 1, 1909. Subject, C. Exercise, stationary bicycle.

Time.	Venous pressure.	Remarks.
3.20	6.5	Control record.
3.23	...	Exercise begun.
3.27	8.5	....
3.35	9.5	....
3.40	12.5	....
3.45	13.5	....
3.50	15.5	....
3.55	17.0	....
4.00	18.5	....
4.10	17.5	....
4.15	18.5	Exercise stopped.

The exercise lasted fifty-two minutes. The venous pressure rose 10 cm. No respiratory distress observed.

Dec. 4, 1909. Subject, Ch. Exercise, stationary bicycle.

Time.	Venous pressure.	Remarks.
9.08	14.5	Control record.
9.09	....	Exercise begun.
9.11	18.5	....
9.13	20.5	....
9.15	19.5	....
9.17	21.5	....
9.19	20.5	....
9.21	20.5	....
9.23	21.5	Subject breathing slower and deeper.
9.26	18.5	....
9.28	17.5	....
9.30	15.5	Exercise stopped.

The exercise lasted twenty-one minutes. The venous pressure rose 7 cm. The maximum rise was not at the end of exercise, however. Indeed the last reading showed a return of the pressure to practically a normal value; there was no period of respiratory distress. It is to be noted that the decrease of venous pressure toward the end of the exercise was accompanied by deep and slow respiratory movements.

This subject served also for the experiment of Dec. 6, 1909.

Nov. 4, 1909. Subject, C. Exercise, stationary bicycle.

Time.	Venous pressure.	Remarks.
11.18	12	Control record.
11.20	..	Exercise begun.
11.23	17	....
11.25	20	....
11.26	22	....
11.28	16	....
11.29	16	....
11.32	20	Exercise stopped. Marked fatigue.

The exercise lasted twelve minutes. The venous pressure rose 8 cm. There was an almost continual increase of respiratory activity. The subject was "sprinting" throughout the experiment.

Dec. 6, 1909. Subject, Ch. Exercise, stationary bicycle.

Time.	Venous pressure.	Remarks.
3.08	2.5	Control record.
3.09	...	Exercise begun.
3.10	9.5	....
3.11	13.5	....
3.12	9.5	....
3.14	11.5	....
3.15	9.5	Respirations deeper and slightly slower.
3.16	11.5	....
3.17	8.5	....
3.18	8.5	....
3.19	8.5	....
3.21	9.5	....
3.22	9.5	Exercise stopped.

The exercise lasted eleven minutes. The venous pressure rose 11 cm. The maximum rise occurred very shortly after the exercise was begun.

This subject served also for the experiment of Dec. 4, 1909.

Dec. 6, 1909. Subject, H. Exercise, stationary bicycle.

Time.	Venous pressure.	Remarks.
3.31	4.75	Control record.
3.35	...	Exercise begun.
3.36	6.75	....
3.38	7.75	....
3.39	8.75	....
3.40	8.75	....
3.42	9.75	....
3.44	12.75	....
3.46	14.75	....
3.48	14.75	....
3.58	....	Exercise stopped.
4.00	14.0	....
4.02	10.5	....
4.05	9.5	....
4.07	8.5	....
4.09	8.5	....
4.10	8.5	....

The exercise lasted twenty-three minutes. The venous pressure rose 10 cm. After the exercise the pressure fell quickly to a constant value, which was, however, 3 cm. above that at the beginning of the experiment.

Dec. 10, 1909. Subject, E. Exercise, stationary bicycle.

Time.	Venous pressure.	Remarks.
3.05	10	Control record.
3.10	..	Exercise begun.
3.12	3	....
3.13	6	....
3.15	9	....
3.20	13	....
3.26	16	....
3.35	16	....
3.40	17	....
3.45	19	....
3.48	21	....
3.50	21	Exercise stopped.
3.54	19	....
3.55	15	....

The exercise lasted forty-five minutes. The venous pressure rose 11 cm.

Dec. 13, 1909. Subject, W. Exercise, stationary bicycle.

Time.	Venous pressure.	Remarks.
3.26	8	Control record.
3.28	12	Control record.
3.35	..	Exercise begun.
3.36	11	....
3.37	20	....
3.39	21	....
3.40	24	Respirations deeper.
3.41	19	....
3.44	15	....
3.45	17	....
3.46	21	....
3.47	22	....
3.48	23	....
3.49	25	....
3.50	26	....
3.51	26	Exercise stopped.

The exercise lasted sixteen minutes. The venous pressure rose 14 cm. There is a transitory fall in pressure coincident with increased respiratory activity.

This subject served also for the experiment of Dec. 1, 1909.

Dec. 13, 1909. Subject, D. Exercise, stationary bicycle.

Time.	Venous pressure.	Remarks.
4.09	14	Control record.
4.10	16	Exercise begun.
4.11	19	....
4.13	17	....
4.14	16	....
4.15	17.25	....
4.17	15.25	....
4.18	17.25	....
4.19	18.25	....
4.20	20.25	Exercise stopped.

The exercise lasted eleven minutes. The venous pressure was 6 cm. Respiration was violent throughout.

These results on man were corroborated on animals. Under morphia-ether anæsthesia stimulating electrodes were placed on the peripheral ends of the cut sciatic nerves as they left the pelvis or upon the peripheral end of the severed dorsal cord. Stimulation was produced with an induced electrical current at the rate of one per second. Arterial pressure was recorded in the carotid. The venous pressure was obtained by passing a long glass cannula into the external jugular until its end lay beyond the junction with the subclavian vein so that the record was not disturbed by the action of the venous valves at this point, or in other cases a similar cannula was passed into the femoral vein until it had passed the valves present at the brim of the pelvis. The cannula and tube connecting with a water manometer were filled with 10 per cent sodium citrate solution. The excursions of the fluid in the manometer were recorded by connecting the latter with a tambour or piston recorder. By this method it was possible to show that the activity of a group of muscles produced a rise of venous pressure. The conditions were not favorable to obtain an extended rise of pressure. The accompanying record (Fig. 1) from a dog shows a sudden sharp rise of venous pressure amounting to about 4.5 cm. at the onset of muscular exercise which is coincident with a considerable increase of respiratory activity. The latter was registered by air transmission, the receiving tambour being strapped to the chest wall. With the cessation of the excessive respiratory activity the venous pressure falls to a point 2 cm. above the control, where it remains throughout the period of muscular exertion. Throughout the stimulation the arterial pressure remains unchanged.

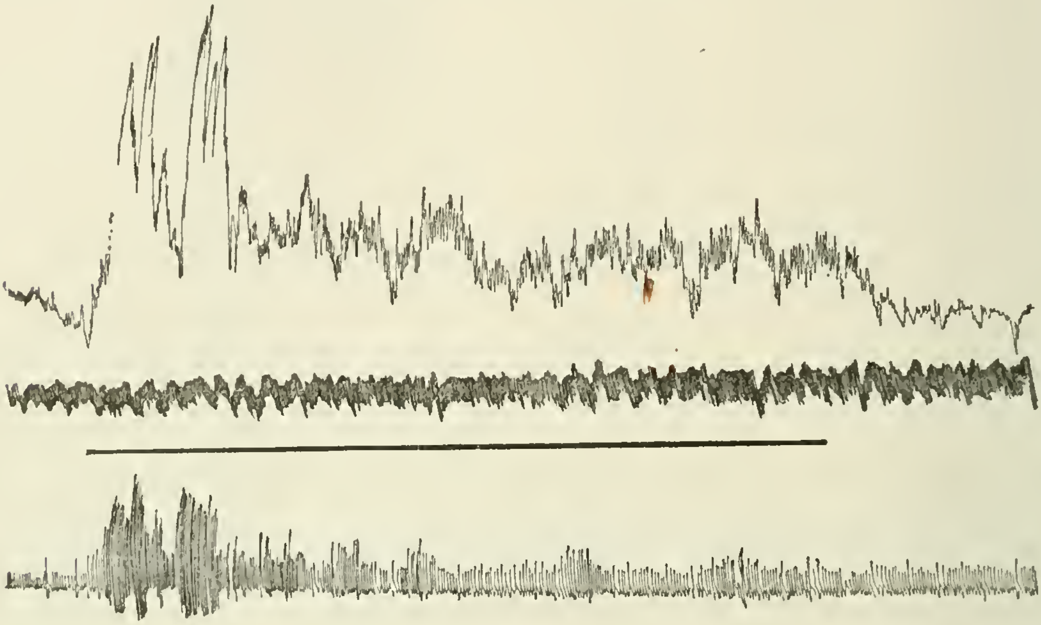

FIGURE 1.—The effect of muscular exercise. First line, venous pressure; second line, arterial pressure; third line, duration of stimulation; fourth line, respiratory movements. The base line for arterial pressure is 46.5 mm. below the signal.

It is obvious that a number of factors must be considered as causative agents in the production of this rise of venous blood pressure during muscular exercise. These may now be briefly considered.

An increase of venous tone due to catabolic products in circulation.— Henderson[5] has advanced the hypothesis that the condition of acapnia is brought about by prolonged forced breathing. The washing out of the carbon dioxide thus produced results in a loss of venous tone which expresses itself primarily as a fall in venous pressure. This condition may be relieved or avoided if the carbon dioxide content of the tissues is held at normal, in which case the tone of the veins is maintained. The rise of venous pressure in muscular exercise may therefore be due to a direct action of catabolic products, and especially of carbon dioxide upon the veins producing an increase of venous tone. Such an explanation would imply a difference in the effect of carbon dioxide upon the veins and capillaries or arterioles, because Bayliss has shown that Ringer's solution saturated with carbon dioxide produces a loss of vascular tone when perfused through the legs of frogs.[6]

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[5] HENDERSON: This journal, 1908, xxxiii, p. xxx.

This objection to Henderson's hypothesis is, however, open to the criticism that the conditions are not analogous. The reaction of vascular tone may vary with the amount of carbon dioxide already present in the tissues. Jerusalem and Starling have shown this clearly for the isolated mammalian heart.[7] Beginning at a carbon dioxide concentration of zero, the efficiency of the heart is improved up to an optimum concentration of from 5–8 per cent of an atmosphere, after which increasing concentrations exert a depressing effect upon the cardiac musculature. It is possible that a repetition of Bayliss' experiments would show a similar difference in the behavior of vascular tone.

It does not seem probable, however, that this explanation is applicable to the exercise rise of venous pressure because the rise of pressure is quite sudden, and the return to normal is equally quick. The asphyxial rise of venous pressure as observed by Plumier [8] in animals with opened thorax, makes its appearance gradually, and does not reach a maximum for a minute or more. The degree of asphyxia thus produced is far greater than in the experiments here discussed. We might expect, therefore, at least as prompt a rise of venous pressure. Furthermore, we should hardly expect a sufficient collection of venoconstrictor catabolic products to produce the rise in pressure observed without a protracted period of discomfort expressed as fatigue.

An expression of venous blood from the limb vessels by muscular massage. — Von Recklinghausen [9] showed that the force of the heart beat was insufficient alone to raise the venous blood from the feet to the heart level in the vertical position. It must be aided by muscular and joint movement which lifts the blood from one valve support to another. The continuous emptying of the veins in the legs when the arterial supply is very free might therefore tend to produce a rise of the general venous pressure. The mechanical effect would be that of excluding a considerable storage space for venous blood. To test this hypothesis, vigorous massage was applied to the legs of a lightly anæsthetized animal. It resulted in a transitory rise of venous pressure. No opportunity was available to make a similar observation on man.

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[6] BAYLISS: Journal of physiology, 1901, xxvi, p. xxxii.

[7] JERUSALEM and STARLING: Journal of physiology, 1910, xl, p. 279.

[8] PLUMIER: Archives internationales de physiologie, 1909, viii, p. 1.

[9] V. RECKLINGHAUSEN: *Loc. cit.*

A decrease in the arterial bed due to splanchnic vaso-constriction by shunting more blood to the venous side. — During muscular exercise in man there is a considerable rise of arterial pressure accompanied by an increase of pulse pressure.[10] This condition could be brought about either by an increase in the unit volume of the heart or by an increase of peripheral resistance. In the latter case the value of the pulse pressure may be shown to vary directly with the peripheral resistance. When the muscles are active, it is quite probable that the blood supply to the splanchnic area is decreased, due to vaso-constriction, for it would be poor physiological economy to increase or even to maintain the blood supply to this area. Such a reaction in the splanchnic area might well cause both the rise of arterial pressure and increase of pulse pressure. It is possible, therefore, that the effect of exercise observed on venous pressure is due to an increase of blood on the venous side, accompanied by an increased resistance to cardiac output. Such an hypothesis is, however, contrary to the present view that the venous system forms a reservoir which is never filled, and consequently the feeding pressure of the heart is maintained constant. But it is to be remarked that every protracted elevation of venous pressure is contrary to such a supposition. Indeed the feeding pressure of the heart must be a constantly varying factor, and it follows that the heart must be able to maintain its function regardless of any normal variation in the venous pressure.

The influence of the factor here discussed is obvious from *a priori* reasoning. We are dealing with a venous plethora caused by splanchnic constriction. The experimental evidence for it is abundantly found in work of Bayliss and Starling.[11] Thus in experiments 5 and 6 of their paper are given the values of arterial and venous pressures before and after splanchnic excitation. The rise of venous pressure following this procedure is apparently independent of the heart rate and arterial pressure as indicated by the following excerpts:

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[10] Lowsley: This journal, 1911, xxvii, p. 446.

[11] Bayliss and Starling: Journal of physiology, 1894, xvi, p. 159.

	Arterial.[12]	Venous.[13]
Before stimulation (one vagus intact)	94	68
During stimulation (one vagus intact)	111–132 [14]	82–86
Before stimulation (both vagi cut)	25	68
During stimulation (both vagi cut)	25–31	84–102

The accommodation of the organism to an increased demand for blood on the part of the active muscles in exercise, brought about by a decrease in the capacity of the splanchnic reservoir, may well, therefore, be a factor in the rise of venous pressure observed during muscular exercise. This accommodation would be aided further by the expression of venous blood by muscular contraction. This factor may be regarded as of considerable importance from a teleological point of view.

An acceleration of the heart rate resulting in a decreased output. — Exercise causes a marked increase in the heart rate. Skiagraphs taken during exercise show a remarkable decrease of cardiac volume.[15] It is not quite clear whether the whole heart or only the ventricles are involved in this change. A record published by Dale, Laidlaw, and Symons [16] would indicate that the effect is chiefly, if not wholly, on the ventricles. The record obtained by a double plethysmograph of a cat's heart shows that when the heart rate is increased by stimulation of accelerator fibres there is a decrease of ventricular and an increase of auricular volume. It is improbable that in this experiment the increase of auricular volume was wholly due to the stimulation. It is much more likely that the ventricular change resulted in a damming back of blood into the auricles which caused a dilatation of the latter chambers. If this is true, the effect should be evident in a rise of venous pressure. An attempt to test such an explanation has been made by accelerating the heart rate in a dog by cutting the vagi. In two animals thus examined there was a rise of venous pressure amounting to 1 cm. of water. In both cases the arterial pressure remained above the control obtained before section of the vagi, which fact excludes the possibility of the rise in venous pressure being due to a loss of arterial tone. It therefore seems probable that acceleration of the heart rate with a resultant decreased unit output, is one factor in the rise of venous pressure which accompanies muscular exercise. If this change in cardiac function is accompanied by an actual decrease in the capacity of the total arterial stream bed converging all the circulatory aids to the active tissues, we might well expect a still greater rise of venous pressure.

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[12] Arterial pressure in millimetres of mercury.

[13] Venous pressure in centimetres of magnesium sulphate. (The values are relative only.)

[14] Heart slowed.

[15] Dietlen: Ergebnisse der Physiologie, 1910, x, p. 598.

[16] Dale, Laidlaw, and Symons: Journal of physiology, 1910, xli, p. 1.

The experimental evidence brought forward in this paper is insufficient to determine what factor or factors are to be considered predominant. It is clear, however, that the heart must be able to function under considerable variations of venous pressure. It is of interest to note that the subjects who exhibited the greatest rise of venous pressure were those in poor physical condition from an athletic point of view. Whether this suggestion has any bearing on the question of athletic over-strain is impossible to state. Lowsley[17] has observed a post-exercise depression in arterial pressure after muscular exertion. If the exertion is excessive, this sub-normal depression of the arterial pressure may be very prolonged, lasting in some instances as much as four hours. It is suggested in Lowsley's paper that the duration of this post-exercise fall in arterial pressure may serve as a criterion as to whether the subject is fit to indulge in a special form of exercise. From the observations of venous pressure here reported, and from the results of Lowsley and others, it is apparent that the heart muscle during violent and protracted muscular exertion, must suffer strain both in lifting the excessive load expressed by the rise of arterial pressure, and in resisting the unusual venous congestion indicated by the rise of venous pressure. The cardiac depression and failure may be due to one or both of these conditions. It is perhaps more probable that acute cardiac dilatation is brought about by venous congestion which interferes with auricular contraction, and so impedes the transmission of the wave of contraction to the ventricle. This probability is strengthened by analogous clinical cases in which the cardiac function is restored by phlebotomy.

The protocols of the experiments indicate that there may be some association between the rise of venous pressure and the respiratory activity. These effects appear to be transient and suggest, therefore, the explanation that they are due to passing changes in the aspiratory action of the thorax, which has no direct bearing on the venous pressure.

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[17] LOWSLEY: *Loc. cit.*

SUMMARY.

The probable sequence of events leading to the rise of venous blood pressure during muscular exercise may be briefly stated as follows:

At the outset there is a local vascular dilatation in the active muscles coincident with an expression of blood from the veins. This results in a prompt rise of venous pressure. The consequent transitory fall of arterial pressure (frequently observed in animals) or an elevation of the temperature of the venous blood [18] is followed by an accelerated heat rate which may, under certain circumstances, interfere with the effectiveness of the heart, and help to cause a stasis of blood in the great veins. Finally, a compensatory vaso-constriction occurs in the great splanchnic area, including the portal vein which shunts the blood to the active muscles. There is, as a consequence, a venous plethora which expresses itself as a rise of venous pressure which continues throughout the period of activity.

NOTE ON THE NEGATIVE PRESSURE IN THE VEINS OF THE FOOT.

The venous pressure upon the dorsum of the foot with the body in a vertical position is, as von Recklinghausen [19] showed, always negative. This means that the driving force of the arterial stream is insufficient to raise the venous column to the heart level. Consequently, the circulatory mechanism would appear to be dependent upon the coöperation of the voluntary muscles of the legs, together with the pump-like action of the joints upon the neighboring veins. This may account, as von Recklinghausen suggests, for the discomfort of standing still for any length of time. If, however, the subject remains in the upright position with as little activity as possible of the limb under observation, the venous pressure of the foot will be found to rise slowly from a negative to almost a positive value. Thus, we have observed under such conditions a rise of from −21 cm. to −1 cm. If, instead of standing, the subject reclines in a steamer chair, the pressure in the foot may reach a positive value. In one experiment the pressure rose from −4 to + 11 cm. This is, however, an unusually marked rise. Flexing of the knee and thigh in the reclining and standing positions always results in a considerable fall in pressure, while voluntary contraction of the muscles without movement of the limb results in a slighter fall. Deep respirations also cause it to fall, and suspended respiration for ten seconds is accompanied by a rise of as much as 15 cm.

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[18] MANSFIELD: Archiv für die gesammte Physiologie, 1910, cxxxiv, p. 598.

[19] VON RECKLINGHAUSEN: *Loc. cit.*

In all of these observations comparative readings showed the pressure of the hand to be 10 cm. or more above that of the foot. It was evident that voluntary exclusion of muscular activity in the leg tended to permit the pressure of the foot to approach that of the hand, but in no case was the approach at all close. If muscular movement compresses and so tends to empty the veins, thus causing the negative venous pressure in the foot, complete exclusion of such movement ought to result in the pressure of the hand and foot becoming equal. We were able to study this in the hospital and operating room. In one case of partial paralysis due to a tubercular lesion of the lumbar cord, the pressure on the hand was found to be + 2, and on the foot −2, a difference of only 4 cm. In a case of complete paralysis, due to a gumma in the lumbar region, the pressure on the hand was + 8 and on the foot + 7. In a case under light morphia-nitrous oxide anæsthesia, the pressure on the foot was −6.5 before the administration of nitrous oxide. Under the anæsthetic for about half an hour it varied from −9 to + 11, the variation being due undoubtedly to alterations in the depth of the anæsthesia and the consequent muscular tone. Readings taken as close together as possible when the patient appeared well relaxed and breathing easily gave for the hand + 7 and for the foot + 10. In a case of deep morphia-ether anæsthesia the pressure rose from a control value of −7 to + 5. A comparative reading of the pressure on the hand at this time also gave + 5.

DISSOCIATION OF INHIBITORY NERVE IMPULSES FROM NORMAL CONDUCTION IN THE HEART BY MEANS OF COMPRESSION.

By WALTER E. GARREY.

[*From the Physiological Department of Washington University, St. Louis.*]

THE question of the nature of the substances which conduct normal impulses in the heart, whether conduction is *myo-dromic* or *neuro-dromic*, is still undecided, and the mode and site of action of the vagus nerve are still in doubt. I therefore present the following simple experiments in the hope that they may tend toward the elucidation of these issues.

The heart of the turtle was used. The method employed in the experiments is to compress the heart tissue between the sinus and the auricle, and, since such compression not only involves the conducting substances of the heart but also the inhibitory fibres from the vagus in their course to the auricle, it was my belief that dissimilar effects would be produced upon these tissues, respectively. This method of experimenting should and does give us direct evidence bearing on the question whether or not conduction in the heart is due to nerve fibres (neuro-dromic), the experiments at least showing that the conducting substance behaves toward compression in a manner which quantitatively differs from the behavior of the intracardiac vagus fibres.

The experiments have been grouped, for convenience of treatment, into three groups. Each group is designed to show in a different way the persistence of vagus effects upon the auricle after the production of complete sino-auricular block. The general method employed has been to take tracings by levers attached to the great veins or sinus and to one of the auricular appendices after the heart has been freed from its attachments except at the base. The coronary nerve and vein are cut in all of the experiments, and a clamp, which is of the modified Gaskell type, is applied, and compression produced sufficient to completely block all impulses passing from the sinus to the auricle. From this point on the procedure varies in the three types of experiments which have been performed.

1. After clamping, time is permitted for the auricles to initiate a rhythm of their own. This may be facilitated by the application of a few drops of *m*/8 sodium chloride solution. When the auricular rhythm is fully developed, a minimal strength faradization is applied to one of the vagus nerves, whereupon we see a typical vagus effect upon auricular contractions; *viz.*, a gradual weakening in the strength of the contraction followed, subsequent to the cessation of vagus stimulation, by gradual return of normal strength of contraction of the auricles. If strong stimulation of the vagus nerve is resorted to, the decrease in the strength of contraction of the auricles is quite sudden, and these contractions may cease entirely, the whole heart becoming quiescent. These results are graphically illustrated in Figs. 1 and 2.

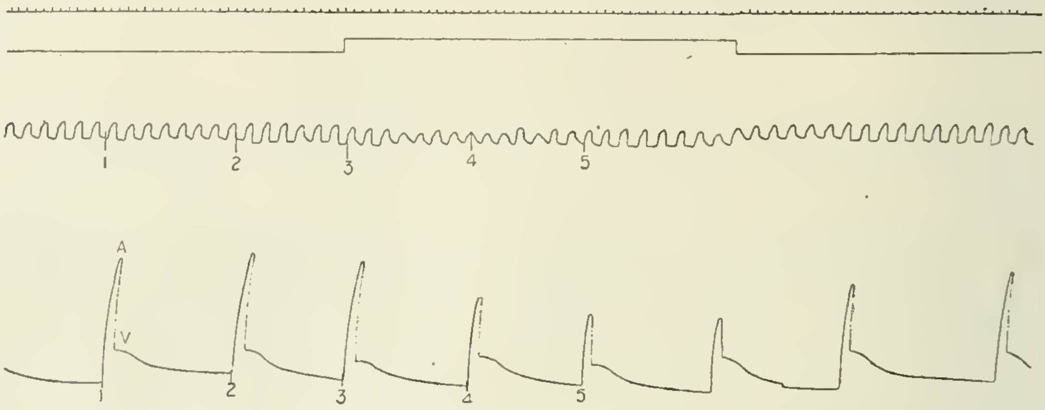

FIGURE 1.

In Fig. 1 the clamp had been gradually tightened at the sino-auricular groove, producing all the intermediate stages up to complete block. The tracings are taken from the sinus venosus and right auricle, which are beating in independent rhythms, as is shown by the study of the time relations indicated by the lines 1, 2, 3, 4, 5, corresponding to the time of auricular beats. The tracing shows that, in spite of the complete block produced by the clamp (a block which was permanent in this case), the vagus can still carry impulses through the clamped area to the auricles. The left vagus was chosen in this case, owing to its relatively slight effect upon the sinus, and a strength of faradization chosen which produced its only apparent effect upon the height of contraction of the auricle, the rate remaining unaffected. The evidence of a vagus effect passing through the clamped area is seen in the decrease in height of the contraction of the right auricle, amounting in this case, to about 40 per cent. Owing to a preponderant homolateral innervation of the heart by the vagus, had the contractions of the left auricle been recorded there would have been shown a still more pronounced decrease in height of contraction. The gradual fall in height with gradual recovery is typical of vagus action on the auricles, as was shown by the work of Gaskell.[1]

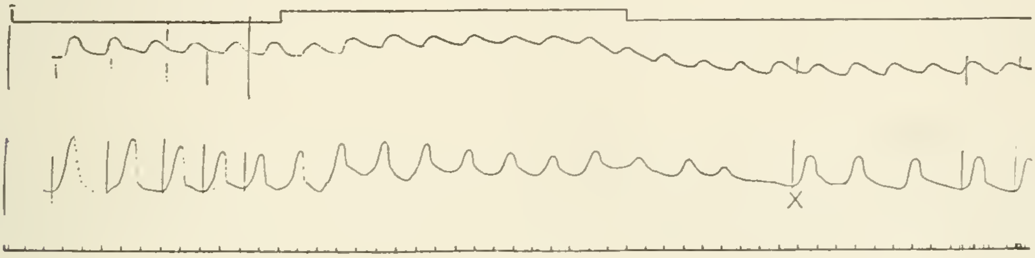

FIGURE 2.

In the experiment represented in Fig. 2 the clamp was so applied that a small portion of the sinus was included on the auricular side of the clamp, which accounts for the comparatively rapid rate of the auricle. The clamp was tightened to a point producing a complete inter-sinus block. The clamp was then removed and the preparation allowed to stand twenty hours before this tracing was obtained. It is evident from the first part of the tracing that as a result of the crushing there exists a complete dissociation of rhythm in the portions separated by the clamped region, and the long interval between the operation and the time when the tracing was taken is a sufficient evidence of complete destruction of the conducting tissue. Nevertheless the vagus effects are distinctly noticeable on the auricular tracing, persisting for a short time and disappearing largely at the contraction marked X. Compression therefore is far less injurious to nerve fibres than to the normal conducting substance of the heart.

2. As is well known, from the results of the Stannius ligature, the spontaneous rhythmic contraction of the auricles is uncertain, and may require a long wait, but in this group of experiments I have re-

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[1] GASKELL: Journal of physiology, iv, p. 43. The question of the homolateral innervation of the turtle's heart by the vagus has been under investigation by the author, and the results will appear later.

sorted to artificial methods of producing regular contractions of the auricles. By means of Engelmann's "Pinzetten" single induction shocks were applied to the apex of the ventricle, the regular intervals being obtained by interruptions from the Bowditch clock, or the ventricles were stimulated mechanically by the point of a needle. By these means reversal of the normal direction of cardiac contraction results, the induced rhythm being a ventriculo-auricular one. This method, therefore, gives a *physiological* stimulation of the auricles and avoids the stimulation of the inhibitory mechanism, which would result from a direct stimulation of the auricles themselves. The arrangement of the sino-auricular clamp permits us, however, to obtain results from stimulation of the vagi. These results are in every sense identical with those obtained in experiments in which the auricles initiate their own rhythm. The tracings shown in Figs. 3 and 4 show these results conclusively.

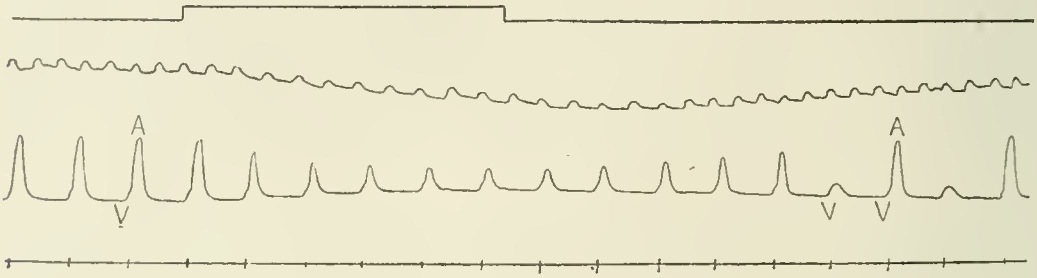

FIGURE 3.

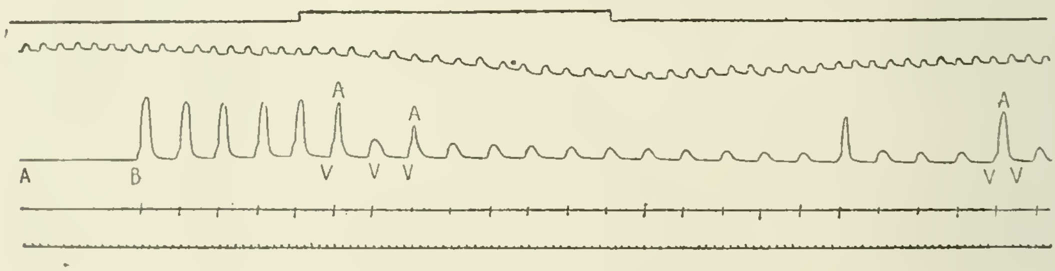

FIGURE 4.

In Fig. 3 the tracings are taken from the right post-caval vein and from the left auricle; the clamp was tightened to complete sino-auricular block and left in place so that throughout the experiment all tissues were being subjected to the compression. The left vagus was stimulated as indicated, with the secondary coil of the Harvard inductorium removed to a distance of 8 cm. Owing to unilateral innervation the right caval veins continue to beat. The beat of the auricle is produced by induction shocks thrown into the ventricle every five seconds, as described above. When the vagus is stimulated by faradization, a sharp fall in the upper auricular portion (*A*) of the contraction *v-a* is seen, with a gradual recovery after cessation of stimulation. Another vagus effect is shown in the right-hand portion of the tracing, *viz.*, a *ventriculo-auricular* block with the production of a rhythm in the ratio $v/a = 2/1$. Conductivity or excitability of the auricles or both attributes are therefore affected in the same manner as the height of auricular contractions by the vagus impulses which pass through the compressed area.

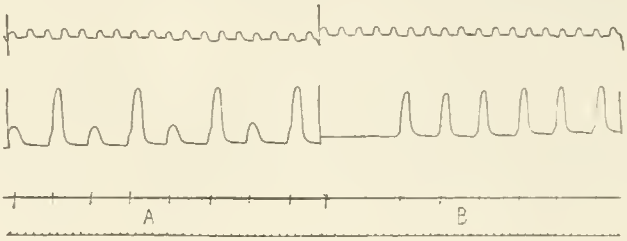

FIGURE 5.

Fig. 4 is a continuation of the tracing in Fig. 3. The first portion (*A-B*) of the tracing shows the efficiency of the sino-auricular block. At *B* the ventricle is again stimulated, and it is to be noted that in the interval the *v-a* block produced by the previous vagus stimulation has disappeared. The left vagus is now stimulated again, but with stronger interrupted shocks (secondary coil at 6 cm.); the effect is immediate, the *v-a* block returns, and the height of the auricular contractions is noted to be decreased. With this stronger vagus stimulation the effects upon the auricle are much more persistent than with weaker stimulation, but gradually disappear. This recovery from the effects of vagus stimulation is shown in Fig. 5; the tracings *A* and *B* being taken after three and seven minute intervals respectively. In the former (*A*) the rhythm has become $v/a = 2/1$, in the latter (*B*) the *v-a* block has disappeared. The complete *s-a* block, due to the clamp, is also shown in the first portion of *B*.

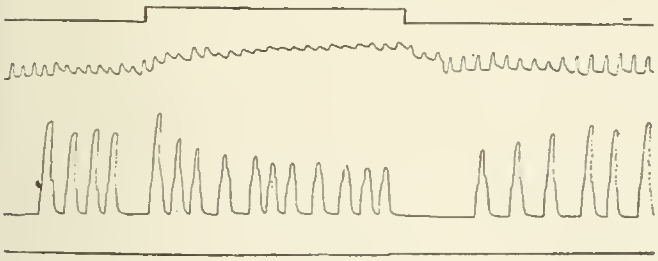

FIGURE 6.

The persistent vagus effects after compression by the clamp between the sinus and auricles may also be seen in Fig. 6. In this case after production of complete *s-a* block the auricles were made to contract by mechanically pricking the ventricle. The stimulation is not perfectly regular, but the fall in height of the auricular portion of the recorded contraction is decidedly marked — from 8 or 10 mm. to 2 mm. The ventricular portion of the contraction is affected only by the stimulation intervals.

3. In the two types of experiments just mentioned the criterion of vagus action has been the height of auricular contraction. The question whether conductivity and excitability decrease also as a result of those vagus impulses which pass through the compressed area has already been mentioned in the description of Figs. 3 and 4, but can be much more strikingly shown in the following way. After the first clamp has been placed between sinus and auricles, as described above, and sufficiently tightened to produce quiescence of the auricles and ventricle, a second clamp is placed at the ventriculo-auricular junction. The amount of compression is graded to a point almost, but not quite, sufficient to produce the first stages of block, then the ventricle is rhythmically stimulated in the method described in the last section. Now, while rhythmically stimulating the apex of the ventricle, either vagus nerve is stimulated with weak faratic shocks. The result is direct and immediate, partial or complete *ventriculo-auricular* block is produced. The degree of block depends upon the strength of the vagus stimulation and continues for some time after interruption of the stimulation of the vagus nerve.

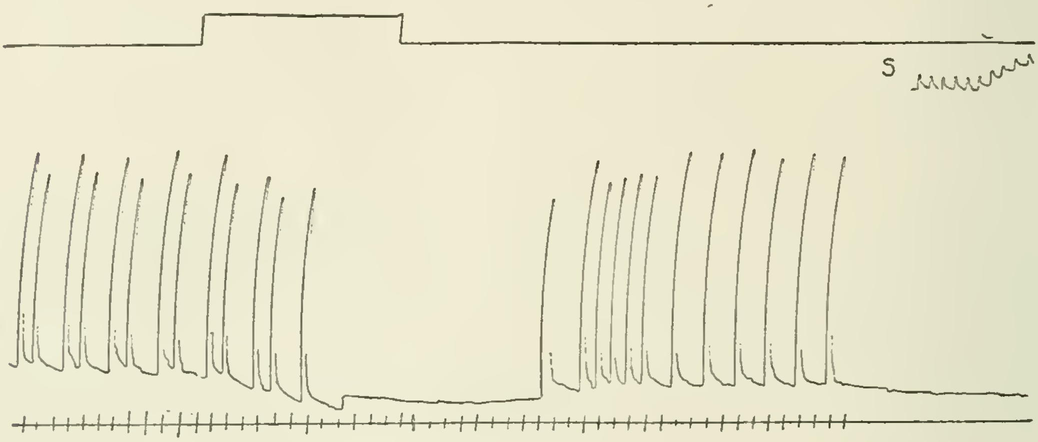

Figure 7

In Fig. 7 the result of this procedure is illustrated. With a very weak stimulation of the right vagus, following a fall in tonus, there is a sudden and complete blocking of all impulses which are passing through the ventriculo-auricular clamped region. The small tracing at *s* is taken from the sinus, and in time relations corresponds accurately with its position relative to the auricular tracing. It was transposed to show the complete *s-a* block and to give a correct idea of the cardiac rate. In this experiment both clamps remained *in situ* and the compression was not relieved while the experiment was in progress.

Fig. 8 represents the same type of experiment as shown in Fig. 7, except that the ventriculo-auricular clamp was removed after compressing to the proper point; thus the tracing shows the ventricular tug as well as the auricular contraction. In both these experiments the ventricle was stimulated every five seconds.

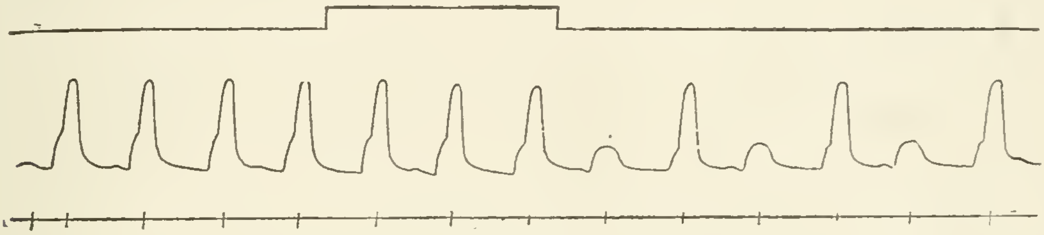
FIGURE 8.

It is not the purpose of this paper to discuss the evidence concerning the much mooted question of the relative importance of muscular or nervous tissue in the transmission of the physiological impulses in the heart; the topic is familiar to every physiologist. It is my desire to present these experiments simply as new evidence bearing on this issue. The evidence is certainly direct and the conclusions perfectly obvious. The clamp, which in all of the experiments is placed between the sinus and the auricle, compresses equally all types of conducting tissue, and it is certainly reasonable to assume that if the conduction can proceed along the vagus nerves when they are stimulated, it should be able to proceed along all other nerves as well. Conversely, if normal physiological conduction from sinus to auricle proceeds along nervous paths, the blocking of these paths should, at the same time, block other nervous paths, including all vagus fibres which pass to the auricle through the clamped area, and through this area only. The experiments considered above show conclusively that this is not the case, and therefore speak directly against the view that physiological impulses pass from sinus to auricle by nerve paths. Of course it may be assumed that the normal conducting tissue may be nervous in nature, but that these nerve fibres may comport themselves differently to compression than do the inhibitory fibres, either as a result of a different constitution or different anatomical structure and arrangement. This assumption, however, is gratuitous and must be put to the experimental test before being seriously considered. The experiments, as conducted above, show conclusively that a degree of compression sufficient to establish a complete sino-auricular block does not interfere in any way with the passage of vagus impulses; they furthermore demonstrate that the degree of compression can be increased sufficiently to completely destroy the normal conducting substances with the consequent production of permanent and irreparable sino-auricular block without producing a destruction of conductivity in the vagus fibres subjected to the same compression. There is, therefore, a marked difference in the behavior between compression of vagus nerve fibres and normal conducting substances in the heart. This fact takes on added significance in view of the recent experiments of Meek and Leaper,[2] who have shown that the degree of compression required to block the passage of impulses in nerve fibres and in skeletal muscle fibres is not markedly different. It seems probable, then, that conduction in the heart must involve a tissue much more sensitive to compression than ordinary motor nerves or skeletal muscles, or some sensitive mechanism of which we are not yet cognizant.

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[2] MEEK and LEAPER: This journal, 1911, xxvii, p. 308.

THE RELATION OF THE SPLEEN TO THE FIXATION OF ANTIGENS AND THE PRODUCTION OF IMMUNE BODIES.*

By ARNO B. LUCKHARDT and FRANK C. BECHT.

[From the Hull Physiological Laboratory of the University of Chicago.]

I. Introductory.

As soon as it was discovered that the normal physiological activity of an animal was dependent on the immune bodies contained in its body fluids which protect it against invasion by disease-bearing organisms, investigators sought to explain the mechanism of antibody production. As a result we have to-day the well-known theories of immunity: the side chain theory of Ehrlich and the phagocytic theory of Metchnikoff. It will be recalled that Ehrlich formulated a definite method according to which antibodies are formed in the living body without specifying any organ or tissue as particularly engaged in their production, whereas Metchnikoff ascribes their production to the direct or indirect activity of the phagocytes (macrophages and microphages).

Of all the tissues of the body the blood possesses the greatest number of polymorphonuclear leucocytes (microphages). And yet Hektoen and Carlson[1] have by transfusion experiments advanced direct proof that the blood takes no direct part in the fixation of antigen (goat's or rat's corpuscles) nor in the production of immune bodies for these corpuscles. What tissue or organ fixes antigen from three to forty-eight hours after intravenous injection?

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* A preliminary report of this work was made before the American Physiological Society at the New Haven meeting in December, 1910; and a brief abstract of the work published in the " Proceedings " of that meeting.

II. LITERATURE.

Concerned as it is with the production of the blood, the hemopoietic system (spleen, lymph glands, bone marrow) has been suspected of also elaborating the various antibodies found in the blood. The experimental evidence which has been obtained by some investigators in support of this supposition has not been confirmed by others.

1. **Splenectomy.** — Bardach[2] found splenectomized animals much more susceptible to the fatal influence of pathogenic organisms or their endotoxins than normal animals. Von Kurlow[3] reported that normal animals were no more resistant towards pathogenic bacteria than asplenic animals. Blumreich and Jacoby,[4] on the other hand, found that splenectomized animals were *more* resistant to pathogenic bacteria than the normal control animals.

According to Kraus and Schiffmann[5] splenectomy had no influence on the production of precipitins (horse serum) or typhoid agglutinins. In a number of cases Deutsch[6] found that splenectomy arrested the development of typhoid agglutinins. Jakuschewitch[7] reported that the serum of splenectomized animals had a greater hemolytic action for foreign corpuscles after immunization than the serum of normal animals which were similarly immunized. Szokalski[8] did not find that splenectomy exercised a retarding influence on the formation of specific hemolysins. In four splenectomized dogs injected with rat's corpuscles Hektoen[9] reported "a lower but otherwise typical antibody curve than is usually the case under otherwise comparable conditions" (Harvey lecture, 1909–1910).

2. **The antibody content of the spleen** was studied at various periods after immunization and compared with the antibody content of the serum and other organs of the same animal. A. Wassermann,[10] Pfeiffer and Marx,[11] van Emden,[12] Jatta,[13] M. Wassermann,[14] and Cantacuzène[15] discovered specific antibodies in the spleen, bone marrow, and lymph glands some days before they appeared in the blood of the animal and concluded that these tissues elaborated them. Rath,[16] Fodor and Rigler,[17] and Deutsch[6] denied that extracts of spleen or other organs possessed a higher titre (typhoid agglutinins) than the serum.

3. **Injury of the hemopoietic organs** by various methods was practised by some in a study of this problem. Benjamin and Sluka[18] report

that exposure of rabbits to the X-rays, which are known to have a deleterious influence on the blood-forming organs, greatly checked or entirely prevented the formation of precipitins.[19] Brezina injected into animals cytotoxic sera for spleen, bone marrow, and lymph glands, and found that such animals did not develop antibodies for *b. coli* in as great a concentration as his control animals.

4. Acute hemorrhage, which *stimulates* the blood-forming organs to marked activity, has been known for some time to cause a considerable increase in the formation of antibodies.[20] This fact has more recently been confirmed by Dreyer and Walker[21] and Hektoen and Carlson.[1]

5. On histological grounds we have the observations of Cantacuzène[22] and Freymuth.[23] The former finds an enormous overproduction of mononuclear leucocytes in the lymph glands, spleen, and bone marrow after the injection of horse serum; the latter reported evidence of increased cellular activity in the bone marrow and spleen on the second and third day after immunization with *b. typhosus*.

III. METHODS.

Assuming with Ehrlich that the organs which fix antigen are also intimately concerned with the production of the specific immune bodies for these antigens and knowing that the antigens (goat and rat corpuscles) are removed from the blood from three to forty-eight hours after intravenous injection, successful transplantation of the spleen from one dog immunized twenty-four hours previously with an intravenous injection of antigen into a normal dog ought to be followed by the appearance of specific antibodies in the blood of the latter *providing* the spleen played any part in the fixation of antigen and production of the immune substances. On account of the technical difficulties involved this direct method of attacking the problem was abandoned, though the operation has been successfully performed by Dr. Carrel.[24]

Operative. — The problem was, therefore, attacked indirectly by the use of the following methods:

1. *Intraperitoneal injection of "immune" spleen.*[6] The spleens of dogs immunized by a single injection of antigen were removed asep-

tically and ground up by a sterile meat grinder. The pulpy mass was suspended in warm physiological salt solution and quickly introduced into the peritoneal cavity of a normal dog. Dogs into whose peritoneal cavities we introduced spleen pulp from normal animals served as controls. The dogs were bled at various intervals for about three weeks. The sera were preserved and tested under the same conditions and on the same suspension of antigen at the end of that period.

The following considerations form the basis for this procedure. If the splenic cells fix antigen, either or both of two things may happen when the "immune" spleen is introduced into the peritoneal cavity of a normal dog. The splenic cells may escape death and give rise to the specific antibodies in the second dog; or the antigen may be split off on the death of the splenic cells and reaching the circulation may again attach itself to suitable receptor and thus stimulate the production of specific antibodies.

2. *Splenectomy.* — If the spleen takes a significant part in the production of antibodies, removal of that organ ought to markedly influence the *rate* and the *extent* of their production. In other words, an animal possessed of a spleen ought to produce the specific antibodies more quickly and in greater ultimate concentration. In all previous work, with exception of the few experiments reported by Hektoen,[9] these two points did not receive the consideration which they deserve in studying this problem.

Dogs were used in all experiments. Following splenectomy various time intervals were allowed for recovery from the effects of the operation before actively immunizing them with a single intravenous injection of goat's or rat's corpuscles; for it was thought that the longer the time allowed for recovery the greater the possibility of other organs vicariously taking up the function of the removed spleen. For each splenectomized animal we provided on the same day a control animal having the same age, weight, and size, and in order to make conditions as comparable as possible performed a laparotomy at which occasion the spleen was temporarily removed from the abdominal cavity and replaced. Under these experimental conditions the differences which were subsequently noted could be unqualifiedly attributed to the fact that one animal was bereft of a spleen; since both had been subjected to anesthesia and surgical manipulations and had synchronously recovered from the debilitating effects of an operation under identical conditions.

Collection, Preservation, and Testing of the Sera. — After immunization the animals were bled daily for the first nine days in order to study exactly the progress of the immunity. In order to get as complete an immunity curve as possible they were also bled on the twelfth, sixteenth, and twenty-first day. The various blood samples were procured by inserting a sterile hypodermic needle into one of the large superficial leg veins. Since we kept the sera three weeks before testing them on the same suspension of antigen, it was necessary to prevent marked deterioration by storing them in a cold chamber at approximately 0° C.

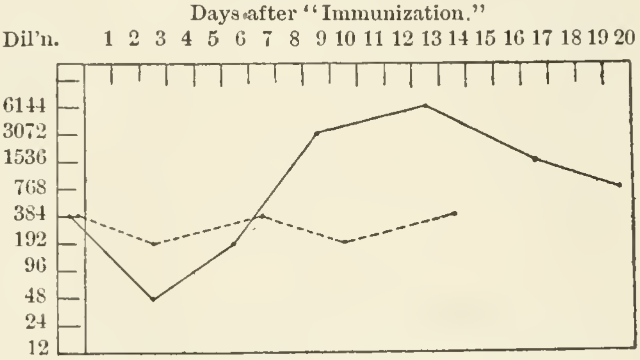

FIGURE 1. — Hemolysins for goat's corpuscles. An emulsified "immune" spleen was injected intraperitoneally into Dog A; a normal spleen was similarly introduced into Dog B. ———— = Dog A. .... = Dog B.

On the day of testing the goat immune sera were heated to 49° C. for thirty minutes to prevent lysis of the goat's corpuscles which would interfere with the agglutinin and opsonin determinations. To determine the extent of lysis the same heated serum was recomplemented by the addition of the proper amount of fresh guinea pig's serum.[1]

IV. RESULTS.

1. **Intraperitoneal injection of "immune" spleen.** — Figs. 1 and 2 show at a glance the results obtained by this method of experimentation.

Fig. 1. Specific hemolysins for goat's corpuscles. Dog A received intraperitoneally the emulsified spleen of a dog immunized twenty-four hours previously with an intravenous injection of 1 c.c. of a 10 per cent suspension of goat's corpuscles per kilo body weight. Dog B received intraperitoneally an emulsion of spleen of a normal animal.

Fig: 2. Specific hemagglutinins for rat's corpuscles. In this experiment Dog A received the emulsified "immune" spleen. Dog B received intraperitoneally ground up bone marrow from the same dog which furnished the "immune" spleen.

In a preliminary report of this work [25] we published a similar result as regards the formation of specific agglutinins for goat's corpuscles.

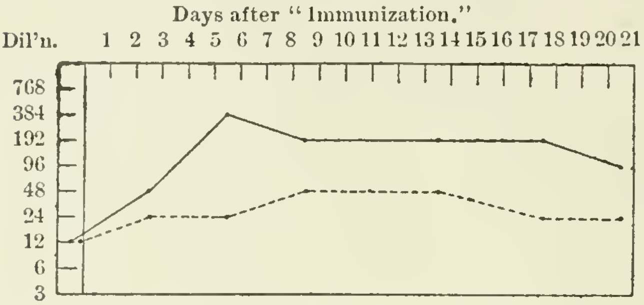

Figure 2. — Agglutinins for rat's corpuscles. An emulsified "immune" spleen injected intraperitoneally into Dog A; "immune" bone marrow similarly introduced into Dog B. ———— = Dog A. .... = Dog B.

In two instances we used a bacterial antigen (*b. typhosus*). The experiments were performed in the following manner. Three days previous to splenectomy Dog A received a large subcutaneous injection of *b. typhosus* killed by heating for half an hour at 60° C. On the fourth day after the injection the spleen was removed aseptically, emulsified, and injected into the peritoneal cavity of Dog B. Dog C received an intraperitoneal injection of 20 c.c. defibrinated blood of Dog A. The two dogs were bled at regular intervals. In one experiment we obtained the following result:

	Days after "Immunization."					
	1	3	6	9	12	21
Dog B	1/20	1/40	1/100	1/100	1/100	1/50
Dog C	1/20	1/20	1/20	1/20	1/20	1/20

A second experiment similarly performed yielded negative results. The results of these experiments demonstrate clearly that the spleen fixes antigen. The mechanism of subsequent antibody production whether by growth of living splenic cells or a second fixation of antigen liberated by the dead or dying splenic cells remains an open question.

The same method has been employed with "immune" lymph glands, bone marrow, liver, and heart muscle. The results were practically negative. In one experiment the introduction of "immune" lymph glands into the peritoneal cavity of a dog was followed by a slight increase in the lysins for goat corpuscles. The result of an intraperitoneal introduction of "immune" bone marrow is recorded in Fig. 2. The rise noted in this experiment is only suggestive of possible results which might follow the injection of a greater amount of "immune" bone marrow than we could procure from one dog.

2. **Splenectomy.** — As above stated, we resorted to the method of splenectomy to (*a*) study daily the rate of antibody formation and (*b*) to get comparative figures on the ultimate extent of immunization in the asplenic and normal animals. In addition, we thought that the longer the time interval allowed for recovery from the splenectomy before immunization the less would be the difference between the control and splenectomized animals in these respects; and that immunization effected immediately after removal of the spleen would be followed by a slower elaboration of the immune bodies than if time were given the organism to adjust itself to the new conditions by either a hyperplasia or an increased activity on the part of the remaining tissue which is to an extent responsible for the production of the antibodies.

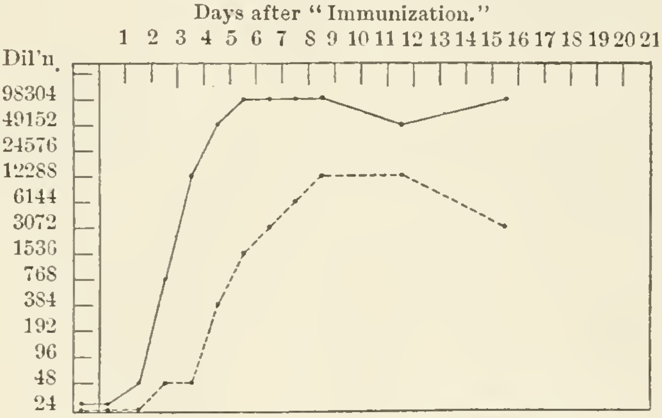

FIGURE 3. — Curves showing difference in rate of formation of lysins from goat's corpuscle and the concentration reached between a splenectomized and a control dog. . . . . = Dog 7, splenectomized. ———— = Dog 8, control.

It would be both useless and tedious to discuss in detail the results obtained from nineteen splenectomized and control dogs which were immunized immediately and on the fifth, eleventh, fourteenth, sixteenth, twenty-first day, and three-quarters of a year after splenectomy and laparotomy. Our own summary which follows will show at a glance what results this method yielded with regard to the particular points under investigation. On the other hand, we thought it advisable to submit the following tables of the individual experiments together with a curve which shows graphically the general nature of our results.

As in the previous charts the figures represent the highest dilution of the serum in which the specific antibodies could be detected. *E. g.*, 6 represents a dilution of 1/6; 1536, 1/1536; 98304, 1/98304.

TABLE I.

Concentration of the Specific Hemolysins in the Sera of 8 Asplenic and 7 Con
Intervals after

Series.	Dogs.	Operation.	Days between operation and immunization.	Norm.			
					1	2	3
I	1	Splenectomy	0	...	...	...	1536
	2	Laparotomy	0	...	...	...	1536
	3	Splenectomy	0	...	...	...	384
	4	Laparotomy	0	...	...	...	384
	5	Splenectomy	0	...	...	...	...
II	7	Splenectomy	5	24	24	24	48
	8	Laparotomy	5	24	24	48	768
III	9	Splenectomy	11	192	192	192	192
	10	Laparotomy	11	48	48	48	384
IV	11	Splenectomy	14	...	...	96	96
	12	Laparotomy	14	...	...	48	...
V	13	Splenectomy	16	...	...	384	768
	14	Laparotomy	16	...	...	192	192
VII	19	Splenectomy	275	...	...	...	6
	20	Laparotomy	275	...	...	...	6

TABLE I.

trol Dogs (subjected to Laparatomy) immunized with Goat's Blood at various
the Operation.

Days after immunization.								
4	5	6	7	8	9	12	16	21
1536	1536	3072	3072	3072	1536	6144	6144	49152
3072	6144	6144	24576	49152	49152	3072	6144	6144
384	192	192	192	1536	1536	3072	1536	3072
1536	24576	12288	98304	12288	24576	12288	1536	1536
12	24	768	768	1536	....	....	....	....
48	384	1536	3072	6144	12288	12288	3072	....
12288	49152	98304	98304	98304	98304	49152	98304	....
192	192	384	384	768	1536	3072	768	....
6144	98304	98304	98304	98304	98304	98304	24576	....
48	48	96	96	....	384	768	768	384
24	192	768	768	....	768	384	....	....
384	1536	6144	49152	....	6144	12288	12288	12288
768	49152	98304	49152	98304	4 152	49152	12288	6144
48	384	768	3072	1536	....	3072	1536	....
384	3072	3072	3072	6144	....	3072	1536	....

TABLE II.

Concentration of Hemagglutinins in the Sera of 10 Splenectomized and 9 Con
Splenectomy or

Series.	Dogs.	Operation.	Days between operation and immunization.	Norm.			
					1	2	3
I	1	Splenectomy	0	..	..	..	6
	2	Laparotomy	0	..	..	..	6
	3	Splenectomy	0	..	..	..	6
	4	Laparotomy	0	..	..	..	6
	5	Splenectomy	0	..	..	..	..
II	7	Splenectomy	5	6	6	6	6
	8	Laparotomy	5	6	..	6	24
III	9	Splenectomy	11	3	3	3	3
	10	Laparotomy	11	3	6	6	24
IV	11	Splenectomy	14	..	..	12	6
	12	Laparotomy	14	..	..	6	6
V	13	Splenectomy	16	..	..	12	6
	14	Laparotomy	16	..	..	6	12
VI	15	Splenectomy	21	..	..	24	24
	16	Laparotomy	21	..	..	24	24
	17	Splenectomy	21	..	..	24	24
	18	Laparotomy	21	..	..	24	48
VII	19	Splenectomy	275	..	..	..	6
	20	Laparotomy	275	..	..	..	24

TABLE II.

trol Dogs immunized with Goat's or Rat's Blood at various Intervals after Simple Laparotomy.

Days after immunization.								
4	5	6	7	8	9	12	6	21
0	0	0	6	6	6	6	6	12
12	48	96	384	192	192	192	96	96
6	12	48	48	48	96	48	24	24
24	48	96	192	192	384	384	192	96
6	6	12	24	48	..	..	..	..
12	12	24	48	48	48	48	48	..
96	38	768	1536	1536	1536	768	768	..
6	6	12	12	96	48	48	24	..
96	1536	1536	3072	3072	3072	1536	768	..
0	0	6	6	..	24	48	48	24
12	24	48	48	..	48	48	..	..
12	6	24	48	..	96	96	48	24
12	48	192	96	..	192	96	96	48
12	..	24	48	..	384	384	192	..
24	96	192	384	..	1536	1536	768	192
48	96	96	192	..	192	384	192	192
384	384	384	384	..	768	1536	384	384
6	12	12	12	48	..	96	24	..
24	48	96	96	96	..	48	48	..

TABLE III.

Concentration of Hemopsonins in the Sera of 10 Asplenic and 9 Control Splenectomy or

Series.	Dogs.	Operation.	Days between operation and immunization.	Norm.			
					1	2	3
I	1	Splenectomy	0	..	..	..	24
	2	Laparotomy	0	..	..	..	12
	3	Splenectomy	0	..	..	..	6
	4	Laparotomy	0	..	..	..	24
	5	Splenectomy	0	..	..	..	..
II	7	Splenectomy	5	3	3	3	3
	8	Laparotomy	5	6	12	12	48
III	9	Splenectomy	11	6	6	6	12
	10	Laparotomy	11	12	12	24	96
IV	11	Splenectomy	14	..	..	0	0
	12	Laparotomy	14	..	..	0	0
V	13	Splenectomy	16	..	..	0	0
	14	Laparotomy	16	..	..	3	3
VI	15	Splenectomy	21	..	..	12	12
	16	Laparotomy	21	..	..	6	24
	17	Splenectomy	21	..	..	12	12
	18	Laparotomy	21	..	..	12	12
VII	19	Splenectomy	275	..	..	..	6
	20	Laparotomy	275	..	..	..	6

TABLE III.

DOGS IMMUNIZED WITH GOAT'S OR RAT'S BLOOD AT VARIOUS INTERVALS AFTER LAPAROTOMY.

Days after immunization.								
4	5	6	7	8	9	12	16	21
24	24	24	48	48	24	6	48	48
12	24	24	48	48	48	24	24	48
12	12	12	24	48	24	24	24	24
24	48	48	96	192	192	96	192	..
6	6	6	24	24	..	..	..	..
3	6	12	24	48	48	48	48	..
48	48	..	192	384	384	96	192	..
12	12	12	12	24	24	48	96	..
96	384	384	768	384	192	192	192	..
0	0	0	3	..	6	6	6	6
0	0	3	6	..	6	6	..	..
6	6	12	24	..	24	48	24	24
6	12	24	24	..	24	48	48	24
12	24	..	48	..	96	96	48	..
48	192	192	768	..	768	768	384	192
12	24	24	48	..	192	192	96	96
24	24	48	96	..	192	384	192	192
6	12	24	24	24	..	48	24	..
12	24	48	48	96	..	48	48	..

Summary of results obtained by splenectomy. — In every instance the control animals developed the specific antibodies more rapidly and in higher concentration than did the corresponding splenectomized animals. We have plotted composite antibody curves of all the asplenic and control animals and for ready comparison have transferred them to the same chart so that the marked difference can be seen at a glance.

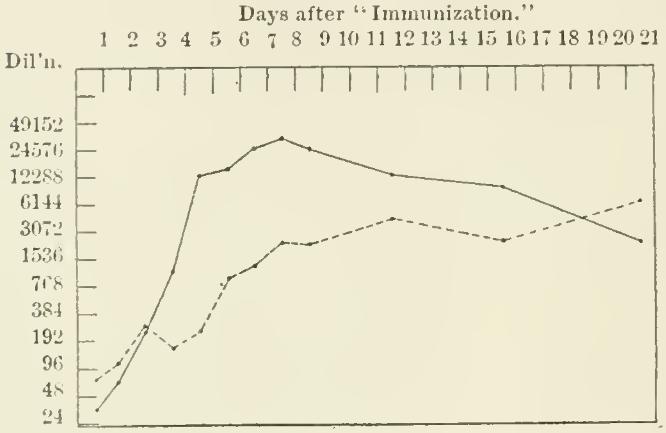

FIGURE 4. — Composite hemolysin curve of 7 control and 8 splenectomized animals, showing marked difference in the rate of formation and in concentration reached. ———— = 7 control dogs. .... = 8 splenectomized dogs.

Fig. 4 shows the composite curves of the hemolysins. We call especial attention to the following two points:

1. The ultimate concentration of the hemolysins in the sera of the control animals (seven dogs) is much higher than in the sera of the splenectomized animals (eight dogs). If the tables are consulted, it will be found that on twenty-seven days the serum of the control animals laked goat's corpuscles in a dilution of 1/24,576 or over. On fourteen of the twenty-seven days the serum of these animals was found to possess lytic action in a dilution of 1/98,304 and on eight days was found active in a dilution of 1/49,152. Comparing these results with the splenectomized animals, we find that on twelve days only was the serum active in a dilution of 1/6144 or over. From the figure we see that the serum of the asplenic animals possessed only one-fourth the titre of the controls at the height of immunity (1/24,576 versus 1/6144).

2. The *rapidity* with which the control animals produced the hemolysins is very striking when it is compared with the rate of formation by the splenectomized animals which normally laked in a higher dilution. In one instance the rise is distinctly abrupt; in the case of the splenectomized dogs the rise to the point of maximum concentration is more drawn out.

Fig. 5 shows the same general results as regards the hemagglutinins. The more abrupt ascent of the curve of the control animals shows how much more rapidly the dogs possessed of a spleen formed the specific agglutinins. On the fifth day the serum of the control animals caused agglutination in a dilution of 1/96. Four days later the serum of the splenectomized dogs was found active in a dilution of 1/48. On that day and on the preceding day the serum of the control animals had eight times that value.

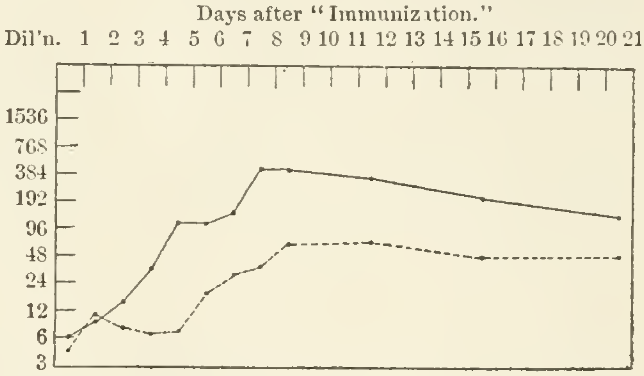

FIGURE 5. — Composite hemagglutinin curve of 9 control and 10 asplenic dogs, showing marked difference in the rate of formation of antibodies and in the ultimate concentration reached. ———————— = 9 control dogs. .... = 10 asplenic dogs.

The composite curves of the hemopsonins show the same relationship between the controls and the splenectomized animals. A glance at Fig. 6 shows how slowly the asplenic animals formed opsonins. On the eighth day of immunity the serum of the control dogs was at least four times as active as the serum of the splenectomized animals.

In many of the individual experiments the height of the immunization was reached by the normal dogs several days earlier than by the asplenic dogs (see Fig. 3. Also hemagglutinins of Dogs 9 and 10, and the opsonins of Dogs 15 and 16).

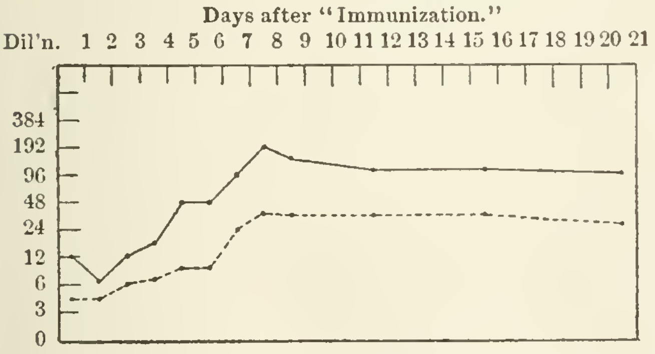

FIGURE 6. — Composite hemopsonin curve of 9 control and 10 asplenic dogs, showing differences in rate of formation of antibodies and in ultimate concentration reached. ———————— = 9 control dogs. .... = 10 asplenic dogs.

These results, which show conclusively that normal animals produce specific hemolysins, hemagglutinins, and hemopsonins more rapidly and in greater concentration than the corresponding splenectomized animals, admit of but one interpretation, namely, that the spleen takes a very active part in the elaboration of these particular immune bodies. The possibility that normally in active immunity the spleen stimulates the antibody-producing organs to activity by means of some internal secretion or hormone does not merit serious consideration, since we have shown that the spleen fixes antigen.

We are not, however, justified in attributing to the spleen the same function when we consider bacterial antigens and their specific antibodies. If the spleen destroys erythrocytes in the normal intact animal, we could have reasoned *a priori* that it would also destroy those foreign corpuscles which the experimenter chose to inject into the blood stream. But, being foreign to the organism, the spleen would react towards this foreign corpuscle (corpuscular antigen) by the overproduction of specific destructive agents — the antibodies in question. The evidence presented in this paper supports this hypothesis. We have, however, no well-founded reasons for supposing that the spleen reacts in like manner towards bacterial antigen. In one experiment we could demonstrate that the spleen fixed bacterial antigen (*b. typhosus*). In another instance, however, we obtained negative results.[11]

It will be recalled that the animals were immunized at various periods after splenectomy and laparotomy to determine whether in the absence of the spleen other tissues or organs would sooner or later take up the function of that organ. We have no evidence on this point which is conclusive. Our methods revealed only *gross* differences in the rate of antibody formation and extent of immunization. Such a compensatory hyperactivity on the part of other organs possibly exists. In one instance (Table VII) we found that a complete compensation had not been in effect three-quarters of a year after splenectomy. We are, nevertheless, inclined to believe from a study of our results that other organs or tissues eventually take up the function of the removed spleen. A great number of experiments with only a slight difference in strength of each successive dilution of the serum will settle this point.

V. Conclusions.

1. **The spleen fixes antigen.** — When an optimum dose of antigen (goat's or rat's blood) is injected intravenously into a dog, the antigen is partly fixed by the spleen; for if the spleen of the dog is removed, emulsified, and introduced into the peritoneal cavity of a normal dog,

the specific immune bodies appear in the serum of the latter. The introduction of normal spleen into the peritoneal cavity is not followed by an increase of the antibodies in the serum of the recipient. The introduction of "immune" heart muscle, liver, bone marrow, and lymph glands did not give positive results.

2. **The spleen is concerned, directly or indirectly, in the immune bodies.** — Asplenic dogs no not produce hemolysins, hemagglutinins, or hemopsonins (*a*) as rapidly nor (*b*) in as high a concentration as the corresponding control dogs.

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ACAPNIA AND GLYCOSURIA.

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I. The Point of View.

GLYCOSURIA is induced by an extraordinarily large number of widely differing conditions. This fact indicates that it is the result of a disturbance of a complex balance involving many factors. With the exception of phloridzin diabetes, practically all forms of glycosuria, whether experimental or clinical, are the result of a hyperglycæmia. The excess of sugar in the blood is in turn, not a fundamental phenomenon, but the expression of diminished ability on the part of the tissues to utilize dextrose. Thus the seat of the complex equilibrium is in the cells, not the fluids, of the body.

The problem of the normal glycogenic function and of experimental diabetes consists in determining the various factors in this equilibrium and defining their relations. The problem of any clinical form of glycosuria lies in discovering which particular factor or set of factors is altered from its normal strength, and how.

One of the most important of the sets of factors involved in the normal balance is the internal or tissue respiration. In his investigations on various forms of experimental diabetes, Underhill[1] noted the casual connection of these conditions with disturbances of breathing. Araki[2] has shown that an insufficient supply of oxygen to the cells (*e. g.*, in CO poisoning) results in a notable glycosuria. Macleod[3] reached the conclusion that in asphyxia it is the excess of CO_2 and not the deficiency of oxygen, which stimulates hepatic glycogenolysis. The work of Edie,[4] and of Edie, Moore, and Roaf[5] seems to demonstrate that an excess of CO_2 in the air breathed apart from oxygen deficiency may result in glycosuria.

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[1] Underhill, F. P.: Journal of biological chemistry, 1905, i, p. 113.

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[3] Macleod, J. J. R.: This journal, 1909, xxiii, p. 302.

We proposed to ourselves the question: Will acapnia also upset the capacity of the tissues to hold sugar? The observations to be here reported indicate that such is the case. One may bring a see-saw to the ground as well by lessening as by increasing the weight on one end. Our observations indicate also that acapnia is a concomitant of some forms of experimental glycosuria in which Edie, Moore, and Roaf have held that hypercapnia must occur.

Glycosuria is known to follow violent emotion in human subjects and to occur in cats which rage at being tied.[6] Henderson[7] has pointed out that the stormy breathing of anger involves excessive pulmonary ventilation. He has likewise shown that ether anæsthesia in dogs without morphin nearly always involves a greater or less degree of acapnia, never hypercapnia. It is noteworthy that a temporary glycosuria is a frequent sequel of ether anæsthesia. Prolonged ether excitement may reduce the CO_2 content of the blood to half the normal amount, and result fatally.[8] These effects, including the glycosuria, are solely due to the excessive breathing induced by ether excitement.[9] We have never found sugar in the urine of dogs which had been brought into deep ether anæsthesia quietly. We believe that both emotional glycosuria and polyuria are frequently the result of acapnia.

Acetone has an influence even more powerful than ether in exciting respiration to excessive activity. When administered to normal animals it has been shown to produce glycosuria.[10] The close association of this substance with the most important clinical form of glycosuria raises the question to what extent acapnia may occur, and what its significance may be, in diabetes mellitus. Acetone is probably a factor in that "nervousness" of diabetics which is in part the subjective expression of their proneness to hyperpnœa.

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[4] Edie: Biochemical journal, 1906, i, p. 455.

[5] Edie, Moore, and Roaf: Biochemical journal, 1911, v, p. 325.

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[9] Underhill: *Loc. cit.*

[10] Albertoni: Archiv für experimentelle Pathologie und Pharmakologie, 1884, xviii, p. 218; v. Jaksch: Zeitschrift für klinische Medizin, 1886, x, p. 362; Ruschhaupt: Archiv für experimentelle Pathologie und Pharmakologie, 1900 xliv, p 127; Müller: *Ibid.*, 1901, xlvi, p. 67.

In diabetic coma a degree of acapnia has been observed more intense than in any other known condition. Magnus Levy[11] found only 3.3 per cent of CO_2 in the blood just before the death of a patient, — less than one tenth of the normal. It is generally assumed that this is not a true acapnia such as results from excessive breathing, but that it is merely a pseudo-acapnia due to the expulsion of CO_2 from the bicarbonates of the blood by organic acids. This view involves, however, a large measure of assumption. To prove it would require simultaneous determinations of the CO_2 tension, CO_2 content, and hydrogen ion concentration $(\overset{+}{H})$ of the blood. No one has yet performed this difficult task.[12]

The line of reasoning usually adopted starts from the fact that (1) Acids are formed in the tissues and pass into the blood in acidosis. (2) Acids liberate CO_2 from bicarbonates. And (3) during acidosis the CO_2 content of the blood is greatly diminished. From these three facts the conclusions are drawn that: (1) In acidosis the acids are produced in an amount sufficient to explain the low content of CO_2 wholly by expulsion. (2) The capacity of the blood to carry CO_2 is destroyed or greatly diminished. And (3) the acidity or $(\overset{+}{H})$ is increased. The facts scarcely warrant any of these conclusions.

Beddard, Pembrey, and Spriggs[13] found that diabetic blood is quite capable of taking up large quantities of CO_2 when exposed to the gas *in vitro*. Evidently the alkalies of the blood even in acute diabetic acapnia are far from being completely neutralized by oxybutyric and other organic acids. These investigators have also recorded the exceedingly important observation that during diabetic coma the CO_2 tension of the alveolar air of the lungs is far *below* normal. Thus they have demonstrated that the acapnia incident to acidosis is mainly a true *low tension* acapnia due to hyperpnœa. From this fact does it not follow that the $(\overset{+}{H})$ of diabetic blood is *below* normal, and that the blood during acidosis is less "acid" than normally, in spite of the neutralization of a part of its alkali bicarbonates by strong organic acids? The pulmonary ventilation of this condition is so excessive that it exhales not only the CO_2 liberated from combination with alkalies, but also a part of that amount which is normally held in the blood in simple solution.

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[11] Magnus Levy: Archiv für experimentelle Pathologie und Pharmakologie, 1901, xlv, p. 389.

[12] For methods see Krogh, Skandinavisches Archiv für Physiologie, 1908, xx, p. 279; Hasselbalch: Biochemische Zeitschrift, 1910, xxx, p. 317. Michaelis and Rona: *Ibid.*, 1909, xviii, p. 317.

[13] Beddard, Pembrey and Spriggs: Journal of physiology, 1904, xxxi, p. xliv.

It is preëminently that portion of carbonic acid which is merely dissolved, uncombined with alkalies, which according to the formulæ of L. J. Henderson[14] determines the $(\overset{+}{H})$ of the blood. This portion obeys the law of Henry. At equal partial pressures of CO_2 in the lungs the carbonic acid dissolved as such will be the same in normal and in acidosis blood. Whichever is exposed to the lower gas pressure will have the less CO_2 in simple solution, and to this extent the lower $(\overset{+}{H})$. As L. J. Henderson has pointed out in his discussion of the neutrality equilibrium of the body, lactic, oxybutyric, and the other diabetic acids when thrown into the blood in moderate amount are almost completely neutralized by alkalies from bicarbonates and phosphates. Short of so intense an acidosis as to absorb practically all of the alkalies of the bicarbonates, the $(\overset{+}{H})$ continues to depend principally just as under normal conditions upon the pressure of CO_2 in the alveolar air of the lungs. In acute acidosis this pressure is much below normal.

Furthermore the body aids itself in neutralizing the diabetic acids by an abnormally large amount of nitrogen in the form of ammonia in the blood and urine. The process of urea formation is, like glycogenolysis, an equilibrium of many factors, of which carbonic acid is one.[15]

From the foregoing considerations it is evident that the excessive respiration associated with many forms of glycosuria cannot be due to an unusually high pressure of CO_2 in the blood. Neither can it be due to a high acidity. It is almost certainly due to the acidosis substances, of which a certain quantity is present in the blood even in health; but in our opinion it is probably the ethereal qualities of

[14] Henderson, L. J.: Ergebnisse der Physiologie, 1909, viii, p. 254.

[15] Macleod and Haskins: Journal of biological chemistry, 1906, i, p. 319.

these bodies and not their acidic influence which, when added to the CO_2 remaining in the blood, excites the respiratory centre to maintain the tension of this gas, and with it the $(\overset{+}{H})$, below normal. That such a conception is a possibility is proven by Henderson's observations that a fatal acapnia may be induced by means of ethyl ether, and by the fact that acetone is an even more powerful respiratory excitant.

A number of investigators have noted that the intravenous infusion of acids exerts a very brief influence upon breathing. The acids are immediately neutralized into their salts; the CO_2 thus displaced is exhaled; and the $(\overset{+}{H})$ depends as before upon the carbonic acid in solution.

The effect of the administration of alkalies in diminishing the dyspnœa during diabetic coma can in like manner be reconciled with our view.[16] If the activity of the respiratory centre is due to the summated influence of the ethereal acidosis bodies and the CO_2 or $(\overset{+}{H})$ in the blood, then diminishing the second term in this sum must reduce their combined stimulating influence in precisely the same manner as that by which alkalies lessen the breathing of a normal subject. It matters not whether the influence of carbonic acid upon the respiratory centre be regarded as depending upon $(\overset{+}{H})$ (Winterstein [17]) or whether, as appears to us more probable, this centre is specifically irritable to CO_2 apart from its acidity.

With such tentative conceptions as these in mind we undertook the experiments here to be reported. They show that acapnia is in fact a constant accompaniment of all the forms of experimental glycosuria studied by us. Upon the more important point, whether or not preventing the development of acapnia will prevent glycosuria, the evidence which we have to report is incomplete. Apparently in some of these conditions such is the case, in others not. We have found it far more difficult than we had expected to administer an atmosphere so rich in CO_2 as to prevent acapnia. As a suggestion to others who may in future work on this or related topics, and as a criticism upon much of the work previously published we would point out that absolutely the only way to find out whether an animal under any experimental conditions is acapnic or hypercapnic is by means of analyses either of the blood or the alveolar air. In many of our own experiments we should have supposed the CO_2 content to be above normal when analyses actually showed it to be much below. Furthermore, as a commentary upon the theorizing of previous workers, it must always be remembered that the only certain criterion of whether the tissues of the body are adequately supplied with oxygen is the demonstration of a considerable quantity of this gas in the *venous* blood. The arterial blood may be saturated and arterial pressure high, yet if the blood stream is small the supply of oxygen will be insufficient for internal respiratory needs and the resulting hyperglycæmia may be mistakenly assigned to other than its true cause.

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[16] *Cf.* Labbé and Violle: Presse medicale, Paris, 1911, xix, p. 292.

[17] Winterstein, H.: Archiv für die gesammte Physiologie, 1911, cxxxviii, p. 167.

Most of the forms of acapnia thus far described by Henderson[18] have been the result of excessive breathing induced by external influences upon afferent nerves. We would point out that perversions of metabolism (*e. g.*, in diabetes and in fever) may produce substances capable of exciting the respiratory centre to abnormal activity. Thus acapnia may be a factor in the pathologic physiology of the diseases of internal medicine no less than in those of surgery.

II. Peptone Glycosuria.

The functional disturbances induced by intravenous injection of proteoses have been studied by a long series of investigators. In addition to the effects of such injections upon the coagulability of the blood and upon arterial pressure, one of the most obvious results of such injections is that upon respiration. The animal exhibits great excitement with struggles, cries, and labored breathing.

It was found by Lahousse[19] in Ludwig's laboratory that in dogs in which the arterial blood contained 33.4 to 39.5 volumes per cent of CO_2 prior to peptone injection, a sample of blood in four minutes after the injection (*i. e.*, when the condition of shock had fully developed) contained only 16.3 to 22.4 per cent CO_2. The oxygen content of the blood was slightly increased. The indications were against the view that the tissue oxidations were diminished. Lahousse observed that the acapnia lasted as long as the condition of shock, and that the CO_2 content of the blood rose again toward normal as recovery progressed.

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[18] Henderson, Y.: This journal, 1910, xxvi, p. 280.

[19] Lahousse: Archiv für Physiologie, 1889, p. 77.

Glycosuria has never, so far as we can find, been observed as a result of peptone injection. The consideration advanced in the previous section led us, however, to expect that it must occur. Our observations confirm those of Lahousse and in addition verify our expectation that glycosuria or at least hyperglycæmia is a sequel of peptone shock and that it is due to acapnia.[20]

They demonstrate that the excessive respiration results in acapnia and is succeeded by a period of subnormal breathing, during which the CO_2 content of the blood rapidly returns to normal. Glycosuria follows. If, however, during the period of excitement excessive pulmonary ventilation is prevented by compelling the subject to breathe through a long tube attached to the trachea, neither glycosuria nor hypercglyæmia occur. Our experiments further indicate that the lowering of arterial pressure following peptone injection is not a factor in the disturbance of sugar control.

Dog of 4 kilos under ether. Urine free from sugar. At 11.07 injected 2.5 gm. Witte peptone in 35 c.c. water into jugular vein. Violent hyperpnœa for a few minutes. At 12.00 the animal had practically recovered. Urine was full of sugar. The NH_4 fraction of its nitrogen was 13.5 per cent.

Cat of 2.1 kilos under ether. At 11.30 injected 1.0 gm. peptone in 20 c.c. water into jugular vein. Hyperpnœa resulted. At 12.21 urine was full of sugar.

Experiment of Oct. 29, 1907. — Dog of 10 kilos. Ether. Injected into femoral vein 3 gm. Witte peptone in 40 c.c. water. Vigorous hyperpnœa.

Time.	Arterial gases.		Blood sugar.	Urinary.	Notes.
	O_2	CO_2	per cent.	NH : N.	
9.50	18.0	30.0	0.16	6.9	
10.08	....	....	....	....	Peptone injected.
10.12	20.5	21.0	0.12	....	
10.55	....	....	....	9.0	Urine full of sugar.
11.30	20.0	26.0	0.26	....	

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[20] Henderson and Underhill: Proceedings of the Society for Experimental Biology and Medicine, 1911, viii, p. 80.

Experiment of Dec. 5, 1907. — Dog, 23 kilos. Ether. Injected 12 gm. peptone in 100 c.c. water. Hyperpnœa resulted.

Time.	Arterial gases.		Venous gases.		Arterial pressure.	Blood sugar.	Notes.
	O_2	CO_2	O_2	CO_2	MM.Hg.	%	
12.40	24.0	40.3	16.6	45.6	145	0.15	Urine free from sugar.
12.44	....	....	...	...	55	...	Peptone injected. Hyperpnœa.
1.00	18.7	30.6	6.6	39.0	45		
1.40	23.0	16.0	16.4	33.4			
3.12	25.0	....	12.6	38.8	95	0.27	Urine free from sugar.

Experiment of Dec. 14, 1907. — Dog, 11.5 kilos. At first chloroformed; then ether. Injected 6.0 gm. peptone. Hyperpnœa resulted. Tube attached to trachea 2 m. long with 13 mm. above.

Time.	Arterial gases.		Venous gases.		Arterial pressure. mm.Hg %	Blood sugar.	Notes.
	O_2	CO_2	O_2	CO_2			
11.45	....	....	...	...	150	...	....
11.46	....	....	...	...	50	...	Peptone injected.
11.55	24.1	32.7	13.6	45.8	...	...	Blood does not clot.
12.00	....	....	...	...	...	...	Tube attached to trachea.
12.44	....	....	...	...	75	...	Tongue pink; breathing deeply.
1.12	23.9	38.5	14.3	51.3	85	...	Blood does not clot.
2.15	....	....	...	...	...	0.17	Urine free from sugar.

III. Piqûre Diabetes.

The Bernard puncture of the floor of the fourth ventricle in a rabbit is usually followed immediately by hyperpnœa. This excessive breathing continues for five or ten minutes, and is succeeded by a compensatory period of subnormal breathing. From half an hour to an hour after the operation polyuria and glycosuria set in. These conditions usually last for four or five hours and then pass off, leaving the animal in apparently normal condition.

The experiments detailed below show that a marked acapnia results from this hyperpnœa. Lahousse [21] has observed that during the time which we have denominated above as the compensatory period the respiratory quotient is low. He regards this fact as indicating an alteration in the nature of the substances undergoing combustion in the tissues. A simpler explanation appears to us to be that, owing to the acapnia, breathing in this period is subnormal. It is sufficient to supply all the oxygen the tissues need, but diminishes the elimination and thus allows reaccumulation of CO_2. During any period when an animal is diminishing its store of CO_2 by hyperpnœa or reaccumulating its normal stock by subnormal breathing the respiratory quotient would be correspondingly increased or diminished. If at the same time the quantity of oxygen absorbed by the lungs continues at an approximately normal rate, the respiratory quotient may afford an entirely misleading indication of the nature of the substances undergoing combustion.

Another object of our experiments was to determine whether the prevention of acapnia would likewise prevent glycosuria after piqûre. For this purpose the rabbits were placed immediately after the operation in a small chamber supplied with an ample stream of air to which known quantities of CO_2 had been added. To our surprise we found it by no means easy to prevent acapnia. With small percentages of CO_2 (5 per cent or less) the hyperpnœa induced by piqûre is sufficient to result in a marked reduction of the body's store of CO_2. Owing to the loss of time involved in learning this, we were obliged, by pressure of other work, to leave many points undecided. Three years have passed without our finding an opportunity to resume the investigation. In the recent paper of Edie, Moore, and Roaf [22] it is argued that hypercapnia is a factor in many forms of experimental diabetes. Our data demonstrate that such is not the case in any of the forms of experimental glycosuria studied by us.

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[21] LAHOUSSE: Archives internationales de physiologie, 1907, v, p. 105. See also LA FRANCA: Zeitschrift für experimentelle Pathologie und Therapie, 1910, vi, p. 1.

[22] EDIE, MOORE, and ROAF: *Loc. cit.*

The data here tabulated show that the prevention of acapnia obviates polyuria and probably to some extent delays and diminishes, but does not entirely prevent, the excretion of sugar after piqûre. It is noteworthy that in one of our control experiments no apparent disturbance of respiration resulted from the piqûre. In this case the appearance of sugar in the urine was delayed and no diuresis was observed.

In all of our experiments the procedure was as follows. The rabbits were anæsthetized with ether. All urine was so far as possible pressed out of the bladder, and tested for sugar. No sugar was found in any case. A sample of blood was drawn from the femoral artery for the gas analyses.[23] Piqûre was then performed. The animal was immediately placed in the ventilated chamber and supplied either with air alone or with air and carbon dioxide of the percentage shown in the table. The chamber was so arranged that samples of blood could be drawn and urine pressed out of the bladder without removing the animal's head and forequarters from the chamber. An attempt to obtain urine in this way was made every fifteen minutes. The data of ten typical experiments are summarized in the table on page 285.

IV. Pancreatic Diabetes.

From the single experiment outlined below it is apparent that when glycosuria is induced by removal of the pancreas at first a notable diminution of the CO_2 content of the arterial blood occurs. The animal breathes excessively and later subnormally. We have no reason to suppose that preventing the development of acapnia would prevent glycosuria or delay the fatal result materially. Even this single experiment shows, however, that pancreatic diabetes does not necessarily involve hypercapnia (*cf.* Edie, Moore, and Roaf on page 283 of this paper). The circulation fails rapidly in all such experiments, as Underhill has frequently had occasion to observe, and the venous blood soon shows by its color an almost entire lack of oxygen. Thus, although the arterial blood may be rich in oxygen, the tissues may be insufficiently supplied because of failure of the circulation. We are not yet prepared to follow the lead of those who hold that deficiency of oxygen is not capable of disturbing sugar metabolism.

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[23] Barcroft and Haldane: Journal of physiology, 1902, xxviii, p. 234. The flasks used by us were three times as large as those of Barcroft and Haldane, and the blood samples were 3.0 c.c instead of only 1.0 c.c.

ARTERIAL BLOOD GASES.

Exp. no.	Before piqûre.		1 hr. after piqûre.		2 hrs. after piqûre.		7 hrs. after piqûre.		Appearance of glycosuria in min.	Polyuria.
	O_2	CO_2	O_2	CO_2	C_2	CO_2	O_2	CO_2		
1[1]	13.5	51.1	...	...	13.3	28.7	13.5	45.7	...	..[8]
2[1]	14.5	58.0	...	...	14.8	37.9	14.5	42.0	30	..
3[1]	12.0	40.0	12.0	26.3	8.5	34.3	12.8	30.0	45	..
4[1]	12.0	38.9	16.4	17.1	..	..	...	...	60	..
5[2]	...	..	13.0	36.0	..	..	..	...	75	0
6[3]	..	..	..	..	..	...	...	...	210	0
7[4]	12.1	50.0	..	..	14.5	47.2	...	...	135	0
8[5]	14.5	45.8	7.3	61.5	9.2	69.5	...	...	75	0
9[6]	16.2	25.7	15.2	44.5	..	..	...	...	60	0
10[7]	..	..	..	..	..	..	...	...	..	..

[1] Breathed air. Hypernœa.

[2] No hypernœa.

[3] Breathed 10 per cent CO_2.

[4] Breathed 15 per cent CO_2.

[5] Breathed 25 per cent CO_2.

[6] Breathed 25 per cent CO_2.

[7] Normal rabbit not anæsthetized; no piqûre, breathed 25 per cent CO_2 for one hour. No trace of sugar appeared in the urine.

[8] .. indicates marked polyuria, and zero indicates no apparent diuresis.

Experiment of February 14, 1908. —

9.45. Dog under chloroform.

10.00. Arterial gases, O_2:19.3; CO_2:42.3 per cent.

11.15. Pancreas completely removed.

11.30. Respiration 30 per minute, and full.

1.10. Urine full of sugar.

1.15. Arterial gases, O_2:19.4 per cent; CO_2:28.6 per cent.

2.00. Tube attached to trachea 2 cm. in diameter and 130 cm. long. Respiration very weak.

2.55. 0.12 gm. morphin sulphate subcutaneously.

3.50. Arterial gases, O_2:23.2 per cent; CO_2:31.0 per cent.

4.45. Urine full of sugar. Respiration almost failing.

5.15. Saline saturated with CO_2 placed in abdomen. Attached tube to trachea. Deep respiration follows.

7.30. Animal stopped breathing.

V. PIPERIDIN DIABETES.

Underhill has observed that when piperidin is painted upon the pancreas a notable hyperpnœa followed by subnormal breathing occurs. Herter[24] had previously demonstrated that painting piperidin upon the pancreas results in a marked glycosuria which persists for several hours. Although we have performed but a single experiment, it appears sufficient to show that hypercapnia is not a factor in this form of glycosuria, but that, on the contrary, the animal develops a notable acapnia. During the period of subnormal breathing which follows the tissues may be insufficiently supplied with oxygen, because of the low content of oxygen in the venous blood.

In the earlier experiments of Underhill it was found that this form of diabetes was prevented when oxygen was given. It is possible that the principal factor in this result was the prevention of acapnia, as the process of breathing into a mask necessarily involves more or less re-breathing. This prevention of acapnia would later obviate also that tendency to failure of respiration and consequent anoxhæmia which were observed by Underhill.

February 25, 1908. — Dog, 15 kilos.

11.30. Chloroform — then ether.

12.00. Arterial gases, O_2:19.9; CO_2:33.7. Venous gases, O_2:15.2; CO_2: 40.9.

12.10. Urine free from sugar; blood pressure, 75 mm. Hg. Piperidin solution —10 per cent — painted on pancreas. Less than 1 c.c. used. Respiration immediately accelerated.

12.15. Arterial pressure, 95 mm.; hyperpnœa.

12.25. Arterial pressure, 110 mm.; coma. Urine free of sugar.

1.10. Arterial gases, O_2: 18.9 per cent; CO_2:27.0 per cent. Venous gases, O_2:11.1 per cent; CO_2: 28.0 per cent.

1.15. Arterial pressure, 130 mm.

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[24] HERTER: Medical news, 1902, xxx, p. 865.

1.45. No sugar in urine. 1 c.c. piperidin painted on pancreas.

2.15. 1 c.c. piperidin 10 per cent painted on [illegible].

2.45. Urine contains sugar.

3.20. Arterial gases O_2:9.9 per cent; CO_2:46.9 per cent. Venous gases, O_2:3.8 per cent; CO_2:57.2 per cent.

3.00 to 3.20. Very feeble respiration. Animal barely alive.

3.25. Slow heart beat; 65 per minute; respiration poor. Arterial pressure varying from 50 to 120 mm. Urine contains sugar.

3.50. Animal died of failure of respiration.

VI. GLYCOSURIA AFTER LAPAROTOMY AND AFTER EXCESSIVE ARTIFICIAL RESPIRATION.

It has been shown by Henderson[25] that when the intestines are handled in a current of warm moist air acapnia develops. The first of the two experiments given below indicates that hyperglycæmia and glycosuria may follow the acapnia.

The most direct procedure for the experimental production of acapnia is excessive artificial respiration. Henderson[26] has found that artificial respiration administered with a hand bellows while the thorax is intact, usually produces little or no acapnia. The elastic recoil of the thorax in expiration under these conditions is so slow that an excessive ventilation is difficult to obtain. It is necessary that the apparatus employed for a dog with intact thorax should not only inject fresh air, but also that it should quickly and forcibly suck out again the otherwise slowly expired air. Excessive ventilation can, however, be obtained merely with a hand bellows after the thorax has been opened. Under these conditions the lungs collapse and expel their air rapidly in the intervals between the strokes of the bellows. This method of inducing acapnia was employed in the second experiment reproduced below. No urine was obtained after acapnia had developed, but the blood sugar exhibited a notable increase.

AERATION OF INTESTINE.

November 19, 1908. — Vigorous bulldog; weight, 7.5 kilos.

10.30. Etherized until 10.45.

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[25] HENDERSON, Y.: This journal, 1909, xxiv, p. 66.

[26] HENDERSON, Y.: This journal, 1910, xxv, p. 322.

11.00. Arterial gases, O_2:23.3 per cent; CO_2:39.6 per cent. Blood sugar, 0.18 per cent; respiration, 42. Pulse, 160; urine free from sugar. NH_4-N fraction : 5.0 per cent.

11.10. Tracheotomized; abdomen opened; intestines aerated; shallow respiration. Very little ether necessary.

12.25. Gases in blood from mesenteric vein; oxygen, 10.3 per cent; CO_2:31.3 per cent.

12.28. Comatose; arterial gases, O_2:17.5 per cent; CO_2:25.8 per cent.

1.00. Respiration, 18; pulse, 170. Good arterial pressure; urine full of sugar; blood sugar, 0.30 per cent; NH_4-N fraction of urine, 8.3 per cent.

December 3, 1907. — Young dog, 10 kilos. First chloroform; then ether; resists anæsthesia.

10.50. Arterial, O_2:17.0 per cent; CO_2:36.1 per cent. Venous, O_2: 14.1 per cent; CO_2:38.4 per cent. Arterial pressure, 150 mm.; pulse, 150. Rapid respiration.

11.15. Thorax opened; rapid and full artificial respiration administered.

11.30. Urine free from sugar.

12.30. No anæsthetic necessary; animal in profound shock. Arterial gases, O_2: 17.2; CO_2: 14.5 per cent. Venous, O_2: 6.9 per cent; CO_2: 33.9 per cent. Intestines relaxed; not irritable; bladder empty.

N. B. { 10.50. Blood sugar, 0.16 per cent.
12.30. Blood sugar, 0.22 per cent.

VII. CONCLUSIONS.

Acapnia is a frequent concomitant of glycosuria or at least of hyperglycæmia both under clinical and experimental conditions. In some artificial forms of diabetes prevention of acapnia obviates disturbance of the sugar-regulating function.

We believe that glycosuria after etherization is due to acapnia, and that traumatic and emotional glycosurias also are usually due to this cause.

The work of previous investigators is here quoted to show (*a*) that in diabetic coma an acute acapnia occurs; (*b*) that this is a true acapnia resulting from hyperpnœa and is not merely due to the expulsion of CO_2 from the bicarbonates of the blood by acids; (*c*) that in acidosis the acidity, *i. e.* $(\overset{+}{H})$, of the blood is probably below normal, instead of above, as usually assumed; (*d*) that the hyperpnœa of diabetic coma is induced by the ethereal, not the acid, acidosis bodies, *e. g.*, acetone.

In conclusion we would point out that it is often impossible to infer the interior conditions of tissue respiration from the external conditions to which an animal may be exposed. The only certain criterion of acapnia or hypercapnia is analysis of alveolar air or blood gases. The only certain criterion of insufficient oxygen supply to the tissues is the demonstration that the venous blood contains no oxygen or only a minimal amount.

SOME ENERGY FACTORS OF THE URINE EXCRETED AFTER SEVERE MUSCULAR EXERCISE.

By HAROLD L. HIGGINS and FRANCIS G. BENEDICT.

[*Contribution from the Nutrition Laboratory of the Carnegie Institution of Washington, Boston, Massachusetts.*]

THE determination of carbon and energy in urine has always been of great importance in total metabolism experiments when a complete balance of intake and output of energy and matter has been desired. In recent years these determinations have become of added interest in that the relationships of the total nitrogen, carbon, and energy in the urine — generally expressed as carbon-nitrogen and calorie-nitrogen ratios — have acquired a pathological significance. The carbon-nitrogen ratio has been studied more than the calorie-nitrogen ratio, but inasmuch as the two are always essentially proportional in different urines, they are of equal significance.

Two important constituents of normal urine — urea and ammonia — give carbon-nitrogen ratios of 0.43 and 0 respectively, and therefore tend to keep this ratio in the total urine low; on the other hand. uric acid, creatinine, and more particularly the other carbonaceous compounds in normal urine raise the ratios. In cases of perturbed metabolism there are almost invariably present in the urine intermediate metabolic products which contain considerable amounts of carbon, and relatively small amounts, if any, of nitrogen. The more prominent of these materials are, perhaps, the organic acids (lactic, diacetic, and β-oxybutyric), acetone, sugar, albumin, amino-acids, and such protein decomposition products as are mentioned by Pregl.[1] The presence of any of these substances in considerable quantity will obviously raise the carbon-nitrogen ratio very appreciably, and it therefore follows that the calorie-nitrogen ratio will be correspondingly increased. Since it frequently happens that the usual study of the

[1] Pregl, F.: Archiv für die gesammte Physiologie, 1899, lxxv, p. 87.

partition of the nitrogen does not throw much light upon the presence of these intermediary products, the importance of a study of the carbon-nitrogen and calorie-nitrogen ratios is readily seen; for by means of these ratios a perturbed metabolism may be easily detected and much information secured with regard to its character, without going into the elaborate estimation of amino-acids, purine derivatives, etc. The determination of these ratios is likewise of especial value when the volume of urine is too small to admit of tests for the individual intermediary products.

Earlier Researches.

In normal urines with ordinary diet, various investigators have found essentially the same ratios. Benedict and Milner[2] report an average carbon-nitrogen ratio of 0.73, and an average calorie-nitrogen ratio of 8.09 from the results obtained in fifty-eight metabolism experiments upon normal individuals in the respiration calorimeter at Wesleyan University. The variations among the different experiments were, in general, very small, the carbon-nitrogen ratio ranging from 0.67 to 0.89, and the calorie-nitrogen ratio from 7.3 to 8.94. Richardson,[3] working with fewer urines, obtained a carbon-nitrogen ratio varying from 0.74 to 1.01 with an average of 0.88. Magnus-Alsleben[4] concludes that with healthy individuals the carbon-nitrogen ratio will not pass beyond the limits of 0.7 and 1.0, regardless of diet. Loewy,[5] Pregl,[6] and others report values with normal individuals essentially within these limits.

Diet seems to have but little effect upon the ratios. Benedict and Milner[7] noticed no appreciable change due to diets containing a preponderance of either carbohydrate or fat; but Tangl[8] found the ratio considerably higher on days when the diet was rich in carbohydrates and poor in fat (average carbon-nitrogen ratio, 0.96; average calorie-nitrogen ratio, 11.67) as compared with days on which the diet was poor in carbohydrates and rich in fat (average carbon-nitrogen ratio, 0.75; average calorie-nitrogen ratio, 9.63). Yet in spite of the fact that the diet on these days was extraordinary, it may be noted that his figures fall within the limits set by Magnus-Alsleben.[9] Moderate muscular work has been shown by both Tangl[10] and Benedict and Milner[11] to raise the ratios but little, if any, over those for rest. Thus, with normal urines, one may conclude that the carbon-nitrogen ratio may vary from 0.67 to 1.0, and the calorie-nitrogen ratio from 7.3 to 11.0.

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[2] Benedict and Milner: U. S. Department of Agriculture, Office of Experiment Stations Bulletin No. 175, 1907, p. 144.

[3] Richardson: Bulletin Mount Hope Retreat Laboratory, 1900. See also Jahresbericht der Tier-Chemie, 1901, xxxi, p. 703.

[4] Magnus-Alsleben: Zeitschrift für klinische Medizin, 1909, lxviii, p. 358.

[5] Loewy: Verhandlungen der physiologische Gesellschaft zu Berlin, 1905–1906, p. 11.

[6] Pregl: *Loc. cit.*

[7] Benedict and Milner: *Loc. cit.*

[8] Tangl: Archiv für Anatomie und Physiologie, 1899, Physiol. Abth. Supplement, p. 251.

Many interesting cases are recorded in which disturbances of these ratios have been found. In fasting Benedict,[12] and Benedict and Diefendorf[13] noted an increase in the calorie-nitrogen ratio as the fast progressed, increasing in one case from a normal ratio of 8.0 to 19.75. Coincident with mountain sickness, Loewy[14] reports an increase in the calorie-nitrogen ratio in four subjects. While the increase was not very large (the average ratios before going up the mountain being 8.58, and on the summit 9.74), it was definitely noted in all four cases. In certain types of fever, also, Magnus-Alsleben[15] found an increase of the carbon-nitrogen ratio, while in others there was a marked decrease. He also reports three cases of the determination of this ratio after severe muscular work, the ratios being extremely high, — 3.262, 1.926, and 1.038.

RECENT RESEARCH AT THE NUTRITION LABORATORY.

In view of the disturbances in the ratios noted by these investigators, it occurred to us that a perturbed metabolism might result from an exhaustive running race. Accordingly, through the kindness of Dr. John H. Cunningham, Jr., we obtained the urines of a number of the contestants immediately after the finish of the recent Marathon Run conducted by the Boston Athletic Association. Some realization of the stress of this race may be gained from the fact that the winner covered the 25-mile (40.26 kilometres) course in less than two and one-half hours, while most of the best runners finished within three hours.

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[9] MAGNUS-ALSLEBEN: *Loc. cit.*

[10] TANGL: *Loc. cit.*

[11] BENEDICT and MILNER: *Loc. cit.*

[12] BENEDICT: Carnegie Institution of Washington Publication No. 77, 1907.

[13] BENEDICT and DIEFENDORF: This journal, 1907, xviii, p. 362.

[14] LOEWY: *Loc. cit.*

[15] MAGNUS-ALSLEBEN: *Loc. cit.*

Although the specific gravity of the different urines was normal, the volume was usually too small to permit the making of many quantitative tests; but we were able to determine in many the carbon, nitrogen, and energy, the nitrogen being determined by the Kjeldahl method, and the carbon and the heat of combustion simultaneously by means of a calorimetric bomb in an adiabatic calorimeter.[16] From the data thus obtained, the carbon-nitrogen ratio, the calorie-nitrogen ratio, and the calorie-carbon ratio were computed. As all of the contestants were unknown to us, it was obviously impossible to control the diet in any way, or to secure any exact data regarding the diet preceding the race; the amount and character of the food taken during the race, if any; the time of the last urination; and the normal constituents of the urine. Without these data, therefore, the total quantities have very little significance, but the ratios may be looked upon as of value.

THE DETERMINATION OF THE HEAT OF COMBUSTION.

In determining the energy of urine, one must remember that bacterial action and decomposition lessen the energy; and further that if urine contains such preservatives as thymol, toluol, or chloroform,[17] a heat determination will be unsatisfactory, owing to the difficulty in ridding the urine of them on drying, the presence of these constituents raising the energy value by an unknown amount. Therefore it is essential to use fresh urine for accurate determinations, unless perhaps either a wholly volatile preservative or a measured amount of a non-volatile one has been added.

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[16] BENEDICT and HIGGINS: Journal of the American Chemical Society, 1910, xxxii, p. 461.

[17] Formalin is also unsatisfactory owing to a residue of paraldehyde on drying.

Tangl added a drop of hydrochloric acid before drying urines in determining the heat of combustion; this served as a preservative and also prevented any ammonia in the urine from volatilizing during the drying. Rubner[18] added a definite amount of oxalic acid to the fresh urine before drying; but we have found salicylic acid to be still more satisfactory by reason of the fact that in addition to preserving the urine and retaining the ammonia, it also acts as a kindler and distinctly aids in the combustion of the dried urine.

In connection with the metabolism experiments with the respiration calorimeter at Wesleyan University, a comparative study was made of different methods for the determination of the heat of combustion. The cellulose absorption blocks of Kellner[19] were tried, but their ready absorption of moisture from the air made their weight and heat uncertain. In duplicate determinations on the same urine, various methods were tried, using different means of evaporation, such as the vacuum desiccator, the water bath, the drying oven at 60°, and by using different preservatives and no preservative at all. But the method which gave the highest heat of combustion, and therefore seemed the best, involved the addition of 50 mg. of salicylic acid to 15 c.c. of urine; the drying then proceeding at as low a temperature as possible. As a result of these tests, the following method, which we have used in this research, has been developed:

An accurately weighed portion of salicylic acid (50 mg.) is placed in an ordinary soft alloy bottle cap,[20] such as is used in milk analysis, and to this, 15 c.c. of urine is added from a pipette. The salicylic acid, on account of its light nature, has a tendency to float upon the surface of the urine, but is readily distributed by blowing gently through the pipette, which is given a rotary motion, thus blowing air against the surface, and forcing the acid into the urine. The bottle caps are then placed in a gentle current of air from an electric fan. In a few hours the liquid will have evaporated to a volume of from 3 to 6 c.c., and it is then carefully transferred into a nickel capsule in which it is later burned in the bomb. The capsules are described by Atwater and Snell,[21] those used in this study being 23 mm. high, 30 mm. internal diameter at the top, and 14 mm. at the bottom, with a capacity of about 9 c.c. of liquid. In transferring the urine, use is made of a swab of ignited asbestos (held in ivory-tipped forceps) to which the last bits of liquid and any dried particles of the urine readily adhere; this asbestos swab, with its adhering material, is then put into the capsule. The capsule is next placed in the draft from the electric fan, and soon the urine is practically dry. During this last drying process, the urine adhering to the upper part of the walls is washed into the lower portion of the capsule with the help of a fresh piece of asbestos wool moistened with distilled water. This method of drying is quicker and much less laborious than the use of a vacuum desiccator: but before finally burning the samples, it is advantageous to put the capsule with the dried urine in a vacuum desiccator for over night. We have found the urine burns readily in the bomb when dried in this manner.

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[18] Rubner: Die Gesetze des Energieverbrauchs bei der Ernährung, 1902, p. 30.

[19] Kellner: Landwirtschaftliche Jahrbucher, 1896, xlvii, p. 297.

[20] An ordinary 100 c.c. evaporating dish may be used, but a metal dish is more advantageous since it readily conducts heat.

TABLE I.

NITROGEN CONTENT OF URINE SAMPLES BEFORE AND AFTER DRYING.

Sample number.	Before drying.	After drying.
	gm.	gm.
1961	1.88	1.86
1963	3.97	3.82
1966	1.88	1.84
1967	1.38	1.36
1968	1.36	1.30
1976	0.67	0.66
1978	1.71	1.68
1979	0.34	0.33
1980	0.64	0.65

To test the efficiency of this method of drying, we determined the nitrogen content of 9 samples of urine dried in this way and found that the average loss in nitrogen as a result of drying and mechanical transferring was less than 2 per cent. The detailed results are given in Table I.

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[21] ATWATER and SNELL: Journal of the American Chemical Society, 1903, xxv, p. 659.

DETERMINATION OF THE CARBON.

The complete oxidation in the bomb of all the carbon of the urine to carbon dioxide allowed us to simultaneously determine the carbon content with the heat. We made use of the method of Fries[22] by which the carbon dioxide formed is determined gravimetrically by passing the gases through soda lime. Obviously there is a correction made for the carbon and heat due to the 50 mg. of salicylic acid present. The heat of combustion of salicylic acid was taken as 5260 calories per gram, the average of a number of determinations made in this laboratory.

ANALYSES OF URINES VOIDED AFTER SEVERE MUSCULAR EXERCISE.

The results of the determinations made on eighteen of the urines collected after the Marathon race, together with the calculated ratios, are given in Table II. An examination of this table will show that the carbon-nitrogen and the calorie-nitrogen ratios of six of the eighteen urines are abnormally high, the ratios of the other twelve being essentially normal, although the tendency seems towards a high rather than a low ratio. A number of tests were made in an attempt to find out the cause of these high ratios.

Ammonia determinations were made on sixteen urines including some of those showing the high ratios, and in but one case (not recorded in the table) was the percentage of nitrogen as ammonia higher than normal, this case showing 9 per cent. Unfortunately the volume of this sample of urine was but 33 c.c., and we were unable to study it further. The low percentage of ammonia and a few tests made directly for β-oxybutyric acid and lactic acid, which showed but a few milligrams in each case, seem to prove conclusively the absence of an appreciable amount of these acid products. None of the urines showed a reducing power greater than normal, each of those

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[22] FRIES: Journal of the American Chemical Society, 1909, xxxi, p. 272.

TABLE II.

ENERGY AND NITROGEN FACTORS OF URINE VOIDED AFTER SEVERE MUSCULAR EXERCISE.

Sample number.	Volume of urine.	Specific gravity.	Nitrogen.	Carbon.	Heat.	Carbon-nitrogen ratio.	Calorie-nitrogen ratio.	Calorie-carbon ratio.
	c.c.		gm.	gm.	Cal.			
1961	202	1.021	1.88	1.702	18.92	0.906	10.06	11.12
1962	160	1.021	1.19	1.224	13.50	1.029	11.34	11.03
1963	365	1.024	3.97	2.813	31.32	0.709	7.89	11.13
1964	240	1.011	1.04	1.272	13.55	1.223	13.03	10.65
1965	41	1.022	0.36	0.546	5.83	1.517	16.18	10.67
1966	204	1.022	1.88	1.731	19.30	0.921	10.26	11.15
1967	132	1.023	1.38	1.318	14.26	0.955	10.33	10.82
1968	101	1.022	1.36	1.193	13.35	0.877	9.82	11.19
1971	80	1.011	0.37	0.515	5.56	1.393	15.03	10.79
1972	355	1.019	2.90	2.128	23.26	0.734	8.02	10.93
1976	89	1.021	0.67	0.568	6.40	0.848	9.55	11.27
1978	99	1.030	1.71	1.379	15.59	0.807	9.12	11.30
1979	71	1.015	0.34	0.433	4.66	1.274	13.71	10.75
1980	58	1.025	0.64	0.689	7.63	1.076	11.92	11.07
1984	142	1.019	1.32	1.264	13.88	0.958	10.51	10.98
1986	138	1.024	1.71	1.519	16.95	0.888	9.91	11.16
1989	142	1.031	1.98	1.745	19.45	0.881	9.82	11.15
1997	93	1.032	1.56	1.311	14.56	0.840	9.34	11.11
Av. .	151	1.022	...	....	....	0.991	10.88	11.02

with a high ratio being tested. Albumen was present in all but four of the thirty-nine urines obtained, occurring in all of those with the high ratios; quantitative tests of some of these latter, made through the kindness of Dr. E. P. Joslin, showed the amounts of albumen to be very small, varying from 0.05 per cent to 0.15 per cent. This would account in a slight degree for the high ratios; but even when the nitrogen and carbon present as albumen are subtracted, the carbon-nitrogen ratio is still high, as may be seen from Table III.

Since there was practically no acidosis and no reducing power, it seems probable that the high ratios in the case of these six men were due to a disturbance of the protein metabolism. Just what substance, in addition to albumen, was responsible for these ratios, we are not prepared to say. Amino-acids may have been present, Loewy having found them in the urine as a result of the effects of high altitudes, and attributed to them the rise in the calorie-nitrogen ratio. But it seems more likely that in this study the high ratios were due to protein decomposition products of higher molecular weight, since these products have a high carbon-nitrogen ratio, while the amino-acids have, for the most part, too low a carbon-nitrogen ratio to give such ratios as were found.

TABLE III.

Carbon-Nitrogen Ratios as affected by Albumen.

Sample number.	Carbon-nitrogen ratio.	Carbon-nitrogen ratio omitting albumen.	Percentage albumen.
1964	1.223	1.205	0.05
1971	1.393	1.244	0.15
1979	1.274	1.132	0.10
1980	1.076	1.014	0.10
1984	0.958	0.942	0.05

The calorie-carbon ratio is seen to be practically the same for all of these urines (minimum, 10.65; maximum, 11.30; average, 11.02). This ratio is very nearly the same as that obtained by Benedict and Milner for a large number of urines (10.96). From our data, therefore, it would seem that if either the carbon or heat of combustion of a urine is known, the other may easily be calculated to within 5 per cent without a direct determination. The reason for the consistently higher value of Tangl (12.33) is not at present apparent. The remarkable constancy of the calorie-carbon ratios leads us to believe that it is of fundamental importance to find a simple, rapid, and accurate method for determining carbon in the urine, since by that means we can obtain not only the energy of the urine, which is of great value, but also the carbon-nitrogen ratio, and from that, of course, the energy-nitrogen ratio. At present, we believe the most accurate method for determining carbon is the one cited in this paper, namely, drying with salicylic acid and combustion in oxygen; but if some volumetric method or wet process could be devised which could be easily and rapidly carried out, it would prove of great assistance to investigators in metabolism and clinical medicine.

SUMMARY.

1. The value of a study of the carbon-nitrogen and calorie-nitrogen ratios is emphasized.

2. A method for determining the heat of combustion and carbon of urine is given.

3. The carbon-nitrogen and calorie-nitrogen ratios of a number of urines passed after a severe, long-distance running race are reported. The values for twelve out of eighteen urines were essentially normal; the remaining six urines gave high ratios, probably due to perverted protein metabolism.

4. In view of the fact that the calorie-carbon ratio is constant, the advantage is pointed out of the development of either a volumetric method or a wet process by which determinations of carbon in urine can be simply, quickly, and accurately made.

THE HEAT OF COMBUSTION OF COMPOUNDS OF PHYSIOLOGICAL IMPORTANCE.

BY ALBERT G. EMERY AND FRANCIS G. BENEDICT.

[*Contribution from the Nutrition Laboratory of the Carnegie Institution of Washington, Boston, Massachusetts.*]

THE importance of a study of the transformations of energy in the animal organism and particularly in the human body has led in recent years to a more careful study of the energy output and a development of animal and human calorimetry.[1] At the present time these transformations of energy have to deal for the most part with the end products of oxidation, and little attention has been paid to the intermediary metabolism. Since in certain cases of metabolism intermediary products are excreted in the urine, it is of importance to note the heat of combustion or the potential energy of numerous products of this type. A glance at the literature shows a number of striking abnormalities in the values assigned, even to the commoner organic compounds. These abnormalities may be ascribed either to errors in technique, fundamental errors in the type of apparatus employed, or the impurities in the substances burned. In more recent years the development of the adiabatic calorimeter has increased in very large measure the accuracy of bomb calorimetry; and thanks to the fundamental investigations of Jaeger and von Steinwehr[2] and Fischer and Wrede,[3] we have well-grounded fundamental values for the heat of combustion of at least two organic products which are obtainable with relative ease, namely, benzoic acid and cane sugar.[4]

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[1] RUBNER has recently introduced the expressive term "biocalorimetry." See TIGERSTEDT'S Handbuch der physiologischen Methodik, 1910, p. 182.

[2] JAEGER and VON STEINWEHR: Verhandlungen der deutschen physikalische Gesellschaft, 1903, v, p. 50.

[3] FISCHER and WREDE: Zeitschrift für physikalische Chemie, 1909, lxix, p. 218.

[4] It is much to be hoped that erelong the fundamental measurements of FISCHER and WREDE may be carried out in an adiabatic calorimeter.

The development of the exceedingly exact resistance thermometer for use in the bomb calorimeter enabled a small temperature rise to be measured with great accuracy, and thus minimized the cooling correction. The adiabatic calorimeter, however, eliminates this cooling correction, and instead of the elaborate time-consuming measurement made by electrical methods with their extremely complex calculations, a good standardized mercurial thermometer may be utilized. The adiabatic condition of the calorimeter precludes any material errors due to radiation and also allows ample time for the thermometer to attain the temperature at the beginning and end of the combustion.

In connection with the researches into the metabolism of matter and transformations of energy in the human body which are in progress in this laboratory, it seemed desirable to have more specific information with regard to the heat of combustion of numerous organic compounds of physiological importance, and hence this investigation was undertaken with the idea of securing pure compounds and making the combustions by means of the exceedingly exact adiabatic calorimeter.

METHOD.

The heats of combustion of these compounds were all made with an adiabatic calorimeter,[5] which has been in successful use for two years and has been repeatedly checked by combustions of pure cane sugar and pure benzoic acid. The hydrothermal equivalent of the apparatus was established by burning known amounts of pure cane sugar and the calorimeter so adjusted arbitrarily as to give the heat of combustion of pure cane sugar of 3957 calories and of pure benzoic acid of 6333 calories. If these fundamental values of cane sugar or benzoic acid should ever be altered by subsequent research, the actual heat of combustion per gram of the substances here reported may be corrected by simple proportion.

As a check upon the purity of the substances, in most instances determinations of the carbon were made in the substance after oxidation inside the bomb calorimeter by means of the method of Fries.[6]

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[5] BENEDICT and HIGGINS: Journal of the American Chemical Society, 1910, xxxii, p. 461.

[6] FRIES: Journal of the American Chemical Society, 1909, xxxi, p. 272.

Practically the only impurity existing in measurable quantities in any of these compounds was moisture; accordingly, in a number of instances, particularly alcohol, acetone, lactic acid, glycerine, etc., where the percentage of water not in the form of water of crystallization was quite considerable, the calculation of the heat of combustion per gram was made on a water-free basis. In the case of three compounds, glycogen, creatinine, and tyrosine, prolonged desiccation demonstrated absence of moisture, but the percentage of carbon as determined was somewhat less than the theory would prescribe. In these cases we have somewhat arbitrarily, it must be admitted, proportioned the heat of combustion on the basis of the determination of carbon. Both specimens of glycogen were probably contaminated with ash, and while no measure of the character of the impurity of the other two substances was made, it is believed that the ratio of the carbon to heat in the most of these specimens is sufficiently constant to justify our procedure, *i. e.*, to assume that the carbon content is an approximate measure of the energy. If, therefore, a substance on analysis yields but 99 per cent of the theoretical carbon, we assume that the heat as measured is but 99 per cent of the true value.

Practically all of the combustions were made at the room temperature within a few degrees of 20° C., and hence represent calories at 20° C. In all instances proper corrections were made for the ignition of the substance by means of the cotton string; for any special igniting substance, such as benzoic acid or sugar, when used; and for the heat of formation of the nitric acid in the process of oxidation.

The substance was usually burned in pellet form, occasionally in loose powder in the bottom of the capsule covered with previously ignited asbestos and the liquid materials such as alcohol, acetone, etc., were burned in glass bulbs. The results as finally presented represent the averages of at least two closely agreeing determinations. Frequently four or more combustions were made with satisfactory agreement.

MATERIALS EMPLOYED.

In all instances the purest kind of material was sought for, but no particular attention was paid to the purification of the materials secured, as we relied solely upon the carbon determinations to indicate the degree of purity.

Cane sugar. — Inasmuch as it was possible to secure very pure cane sugar and the purification of benzoic acid is not very difficult, these two compounds were used as the basis for determining the hydrothermal equivalent of the bomb. The cane sugar was that furnished by the United States Bureau of Standards, and was especially prepared for the standardization of the calorimetric bomb. Repeated determinations of carbon showed that this material was of the highest grade of purity.

Benzoic acid. — ($C_7H_6O_2$). This product was supplied by Kahlbaum. The carbon determinations which were made repeatedly showed the highest degree of purity.

Dextrose. — ($C_6H_{12}O_6$). Kahlbaum. In addition to the carbon determinations, the solutions of this material were carefully controlled by determining the rotation in an accurate polariscope, all tests indicating the highest degree of purity. Analysis showed 39.90; theory, 39.98 per cent.

Levulose. — ($C_6H_{12}O_6$). Kahlbaum. The carbon determinations showed 39.52 per cent; theory, 39.98 per cent.

Lactose. — ($C_{12}H_{22}O_{11}+H_2O$). Merck. Two determinations of carbon showed 40.00 and 40.06 per cent, respectively; theory, 39.98 per cent.

Maltose. — ($C_{12}H_{22}O_{11}+H_2O$). Kahlbaum. Carbon found 40.12 per cent; theory, 39.98 per cent.

Glycogen. — ($C_6H_{10}O_5$). Specimen A. This was furnished through the kindness of Professor Ernst Weinland of Munich, and was prepared from ascaris. Carbon found, 43.24 per cent and 43.45 per cent; theory, 44.42 per cent. This specimen gave 4212 calories per gram, calculated on the carbon per cent.

Specimen B. This was kindly furnished by Professor L. B. Mendel of Yale University, who stated that it represented the purest specimen at the time in the laboratory, but called attention to the ash content. Carbon, 42.44 per cent and 42.34 per cent. This specimen gave 4241 calories per gram, calculated on the carbon per cent.

Asparagine. — ($C_4H_8N_2O_3+H_2O$). Kahlbaum. Carbon found, 31.96 per cent; theory, 31.96 per cent.

Aspartic acid. — ($C_4H_7NO_4$). This material was furnished through the kindness of Dr. Thomas B. Osborne of the Connecticut Agricultural Experiment Station, New Haven, Conn. It was in the form of well-defined crystals and of a high degree of purity, as the analysis showed. Carbon, 36.00 per cent found; theory, 36.06 per cent.

Allantoin. — ($C_4H_6N_4O_3$). This is identical with the allantoin used so extensively by Professor F. B. Underhill in his recent researches, and was supplied us through the courtesy of Professor L. B. Mendel of Yale University. Carbon found, 30.10 per cent and 30.31 per cent; theory, 30.34 per cent.

Alanine. — ($C_3H_7NO_2$). This substance was likewise furnished through the kindness of Dr. Osborne. Carbon found, 40.77 per cent and 40.62 per cent; theory, 40.40 per cent.

Creatine. — ($C_4H_9N_3O_2$). A specimen of the highest degree of purity was supplied by Professor Otto Folin of the Harvard University Medical School. Carbon found, 36.59 per cent and 36.50 per cent; theory, 36.59 per cent.

Creatinine. — ($C_4H_7N_3O$). The specimen was kindly furnished by Dr. Folin. Carbon, 41.99 per cent and 41.90 per cent; theory, 42.40 per cent.

Cystin. — ($C_6H_{12}N_2S_2O_4$). Specimen furnished by Dr. Folin. The preparation from which the specimen was taken has been analyzed repeatedly[7] and shown to be exceedingly pure.

Glutaminic acid. — ($C_5H_9NO_4$). Specimen furnished by Dr. Osborne. Carbon found, 40.64 per cent and 40.63 per cent; theory, 40.79 per cent.

Glycocoll. — ($C_2H_5NO_2$). Kahlbaum. Carbon found, 32.11 per cent; theory, 31.96 per cent.

Hippuric acid. — ($C_9H_9NO_3$). Kahlbaum. Carbon found, 60.37 per cent and 60.15 per cent; theory, 60.30 per cent.

Tyrosine. — ($C_9H_{11}NO_3$). The specimen was furnished by Dr. Osborne. Well crystallized. Carbon found, 59.08 per cent and 59.04 per cent; theory, 59.60 per cent.

Urea. — (CH_4N_2O). Kahlbaum. Carbon found, 19.99 per cent; theory, 19.97 per cent.

Uric acid. — ($C_5H_4N_4O_3$). Kahlbaum. Carbon found, 35.48 per cent and 35.48 per cent; theory, 35.67 per cent.

Alcohol. — (C_2H_6O). Obtained from Merck, of the quality known as "absolute" alcohol. Specific gravity at 15.6° C., as determined by a Squibb pyknometer, was .79794. Carbon analysis showed 51.10 per cent against 52.06 per cent theory. The heat result is calculated with the carbon determination as a basis.

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[7] Benedict, S. R.: Journal of biological chemistry, 1909, vi, p. 371; W. Denis: *Ibid.*, 1910, viii, p. 402.

Acetone. — (C_3H_6O). Merck. Carbon found, 61.36 per cent and 61.35 per cent; theory, 62.02 per cent.

β-oxybutyric acid. — ($C_4H_8O_3$). Kahlbaum. The commercial product was racemic. Carbon found, 45.67 per cent, 45.63 per cent, and 45.65 per cent; theory, 46.11 per cent.

Lactic acid. — ($C_3H_6O_3$). Kahlbaum. Specific gravity, 1.21. The hygroscopic nature of this material resulted in large differences in the carbon determination, the three values found being 34.09, 39.01, and 35.38 per cent, respectively; theory, 39.96 per cent. That these variations in percentage were due wholly to water is shown by the fact that the heat of combustion determined on these materials when calculated on the water-free basis, agreed perfectly in all cases.

Glycerine. — ($C_3H_8O_3$). This material was obtained from the J. T. Baker Chemical Co. The manufacturers' analysis stated that the water was the only impurity. Carbon found, 37.67 per cent and 37.46 per cent; theory, 39.09 per cent.

Palmitic acid. — ($C_{16}H_{32}O_2$). Kahlbaum. Carbon found, 74.82 per cent; theory, 74.91 per cent.

Stearic acid. — ($C_{18}H_{36}O_2$). Merck. The material was re-crystallized by the addition of water to a warm alcoholic solution, and then dried in a vacuum desiccator. Carbon found, 75.79 per cent and 76.19 per cent; theory, 75.91 per cent

Oleic acid. — ($C_{18}H_{34}O_2$). Kahlbaum. Carbon found, 76.44 per cent and 76.56 per cent; theory, 76.51 per cent.

SUMMARY OF RESULTS.

An abstract of all the results is presented herewith in tabular form. Inasmuch as these substances are selected on account of their physiological importance, it seems best to give the results on the basis of constant pressure[8] rather than of constant volume.

Owing to the differences in the type of apparatus used and the development of technique, it seems hardly advisable to enter into an

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[8] BERTHELOT: Thermochimie, 1897, ii, p. 30.

HEATS OF COMBUSTION.

Substance.	Formula.	Heat combustion at constant pressure.	Substance.	Formula.	Heat combustion at constant pressure.
		cal.			cal.
Dextrose . .	$C_6H_{12}O_6$	3739	Glycocoll . .	$C_2H_5NO_2$	3110
Levulose . .	$C_6H_{12}O_6$	3729	Hippuric acid . . .	$C_9H_9NO_3$	5660
Lactose . . .	$C_{12}H_{22}O_{11}+H_2O$	3737	Tyrosine . .	$C_9H_{11}NO_3$	5915
Maltose . .	$C_{12}H_{22}O_{11}+H_2O$	3776	Urea	CH_4N_2O	2528
Glycogen . .	$(C_6H_{10}O_5)$	4227	Uric acid . .	$C_5H_4N_4O_3$	2737
Alanine . . .	$C_3H_7NO_2$	4401	Acetone . .	C_3H_6O	7429
Allantoin . .	$C_4H_6N_4O_3$	2584	Alcohol . . .	C_2H_6O	7104
Asparagine .	$C_4H_8N_2O_3+H_2O$	3065	β-oxybutyric acid	$C_4H_8O_3$	4693
Aspartic acid	$C_4H_7NO_4$	2882	Lactic acid .	$C_3H_6O_3$	3615
Creatine . .	$C_4H_9N_3O_2$	4240	Glycerine . .	$C_3H_8O_3$	4323
Creatinine .	$C_4H_7N_3O$	4988	Palmitic acid	$C_{16}H_{32}O_2$	9318
Cystin . . .	$C_6H_{12}N_2S_2O_4$	4137	Stearic acid	$C_{18}H_{36}O_2$	9499
Glutaminic acid	$C_5H_9NO_4$	3662	Oleic acid	$C_{18}H_{34}O_2$	9423

extensive comparison of these results with those of earlier writers. It is hoped, however, that they may be of value in computing the energy transformations in experiments made either with or without the respiration calorimeter. While the energy value of normal urine may be approximately obtained from the nitrogen content, yet in certain cases of pathological urine, as, for instance, in diabetes, one should know that for every gram of β-oxybutyric acid excreted per day there is a loss of 4693 calories; this must be ultimately taken into consideration with fully as much care as is now customary in computing the energy lost in the urine through the sugar excreted.

ON THE FUNCTIONS OF THE CEREBRUM: CONCERNING THE LATERAL PORTIONS OF THE OCCIPITAL LOBES.

By SHEPHERD IVORY FRANZ.

[*From the Laboratories of the Government Hospital for the Insane, Washington, D. C.*]

SINCE the early experiments on the occipital lobes it has been believed that these parts of the brain are connected with the eyes and that they subserve certain visual functions. Although not distinctly stated, it has been assumed that the occipital cortex acts solely in connection with visual processes, and that the differences in a real localization of this function in man and in animals are due to differences in cerebral characteristics. That the latter is so cannot be doubted, but, on the other hand, the assumption of a unified function for the whole of the occipital cortex appears to be more open to question.

Recent histological studies have shown that the occipital lobes are not anatomically single, and that, in fact, they may be divided into two or three areas with cell and fibre laminations differing from each other.[1] The most generally accepted division is into two areas. One of these extends along and into the calcarine fissure, and crops out for a short distance on the lateral aspect of the hemisphere in man, but in the monkey includes most of the area bounded by the inferior occipital and the parieto-occipital fissures. The other surrounds the first area, extending about 0.5 to 1 cm. beyond it. The first area has been called by Bolton "visuo-sensory," and the second "visuo-psychic," it being supposed that the larger area (especially that along the calcarine fissure in man) has a direct correspondence with the retinal segments and is concerned with visual sensations, and that the smaller, surrounding area is concerned with the elaboration or association of the sensory processes. Although direct physiological or clinical evidence is lacking, it has been concluded that the similarities in structure of the different parts of the visuo-sensory cortex indicate a sameness in function and that the dissimilarities of the visuo-sensory and the visuo-psychic areas necessarily mean functional differences.

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[1] See especially K. BRODMANN: Vergleichende Lokalisationslehre der Grosshirnrinde in ihren Prinzipien dargestellt auf Grund des Zellenbaues. Leipzig Barth, 1909. Pp. 334.

It will be remembered that Ferrier, Goltz, Luciani, Munk, Schäfer, and many others investigated this general region of the cortex by physiological methods and that in general they agree that lesions of the occipital lobes are accompanied by losses of visual ability. This general conclusion has been supported by the correlation of visual defects and cerebral changes in man by many investigators. Henschen in particular, has localized along the calcarine fissure the areas concerned with visual sensations or perceptions and has supposed that there is an almost accurate representation of the retina in this part of the cortex. It will also be remembered that upon stimulation of parts of this region movements of the eye and of associated parts have been observed, and several investigators have correlated the movements with definite cortical areas. Although part of the occipital cortex has been called visuo-psychic, facts which would warrant this conclusion are very few and those obtained on animals are of no value. In an area adjoining the occipitals visual speech function has been located by some, but others have located this function in the angular gyrus, which, it will be remembered, was believed by Ferrier to have the visual functions assigned by others to the occipital lobes.

Because of the dissimilarities in structure of these two closely connected areas, and because of the equivocal nature of the functional evidence in differentiation, it seemed advisable to attempt to discover a possible physiological difference. Especially because of the differences in explanations of the schools of Goltz and Munk, it was deemed advisable to learn how a monkey deprived of part of the occipital visuo-sensory cortex would behave and to compare these reactions with those of the animals deprived of the so-called visuo-psychic cortex and with those of a normal animal. As it progressed, the work developed along different lines and the present communication deals exclusively with the so-called visuo-sensory cortex.

For the determination of the visual processes, in addition to the usual method of causal observation, I employed a method similar to that used by me in former work on the cerebrum.[2] This method, in brief, consists in the establishment of a definite association and the determination of the presence or absence of this association after a certain part of the nervous system is destroyed. In the present series of tests the animals were taught, or acquired, visuo-motor associations. Pieces of bread, of equal sizes, were dipped into colored solutions (Congo Red, Toluidin Blue, Methyl Orange, Methyl Orange and Smargd Grün), making the breads respectively red, blue, yellow, and green. The red and blue breads were, furthermore, sweetened by the addition of saccharin, and the yellow and green were made bitter by the addition of quinine. These breads, in pairs, by threes, or by fours, were presented to the animals, and within a few days (thirty or forty tests) the animals learned to discriminate the sweet (red or blue) from the bitter (yellow and green) breads.

After the visuo-motor association was formed a part of the visuo-sensory cortex was destroyed by an electric thermo-cautery, or a part of the occipital pole was separated from the remainder of the brain by a frontal incision. After the death of an animal the brains were photographed, and there was made a careful histological examination of the portion affected by the lesion and of the neighboring areas. The degenerated areas (determined by microscopical examination) were marked on tracings of the photographs, and the figures with this article are reproductions of the reconstructions.

Eight animals were used in this work, in seven of which the lateral portions of the visuo-sensory cortex were destroyed, and in one of which the similar type of cortex along the calcarine area was affected. Brief case histories of only two animals will be given here.[3]

Monkey 1. *Operation, November* 27, 1909. — An attempt was made to sever both occipital lobes from the rest of the cerebrum, the cuts being made posterior to the parieto-occipital fissure. During a few hours after the operation it was found that the pupils were widely dilated. The attention of the animal was easily attracted, and it took pieces of food and other materials placed on the floor of the cage or held in the observer's hand. The movements which were made were, however, not accurate, and in taking food which was held outside of the cage in which it was placed the animal usually placed its hand from 2.5 to 5 cm. from the food. These movements were slow and indecisive and quite unlike those of the normal animal. In making these movements the arm at times moved toward the food or in the direction of the food, but when the hand was about 7 or 8 cm. distant from the object an additional adjustment was made so that the food could be grasped. It appeared that the initial adjustment was inaccurate, that the distance or the direction was not properly perceived, but that after the initial part of the movement the discrepancy was appreciated and the second adjustment was made. It should be noted that even though the second adjustment was made this did not always enable the animal to reach the food, and at times the fruit or nuts were obtained only after groping.

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[2] On the functions of the cerebrum: The frontal lobes in relation to the production and retention of simple sensory-motor habits. This journal, 1902, viii, pp. 1–22. On the functions of the cerebrum: The frontal lobes, N. Y., The Science Press, 1907, pp. 64.

[3] For fuller details of these and of the other six cases the reader is referred to a forthcoming monograph: On the functions of the cerebrum, The occipital lobes. By Shepherd Ivory Franz with the co-operation of G. R. Lafora.

That the visual ability (*per se*) was not affected was evidenced by the fact that the animal made grasping movements for certain kinds of food and not for others. It selected certain kinds of fruit for which it had shown a liking previous to the operation, and it disregarded pieces of bread (of which it was not particularly fond) when they were presented in a series in which fruit or nuts were being given him. This animal previous to the operation had learned to discriminate yellow (bitter) and red (sweet) breads, and two days after the operation in similar tests no mistakes were made.

Observations of this animal continued for a period of about four months. During the early days following the operation the only defect that could be determined was the inability to make accurate adjustments in the selection of food. After a week it was almost impossible to determine any defect even in the motor adjustment. Since the operation had produced none of the marked visual alterations noted by most previous observers, and since the areas affected by the section were not easily determinable *ante mortem*, a second operation was made. In the intervening period of four months the animal was not practised much on the discrimination of the colored breads, but a sufficient number of tests was made so that the habit was not lost.

Second Operation, April 1, 1910.—At the second operation the cortex on the lateral aspects of both occipitals was burned with the cautery. For some time after this operation the animal was inactive, but in about two days returned to its normal degree of activity. The movements, however, were awkward and slow; food which was presented was sometimes taken and sometimes not taken, and when the movements of grasping were made these were inaccurate. The animal made a few mistakes in the selection of the yellow breads, but these were judged to be due to inaccuracies in movement more than inaccuracies in perception, for in these tests both pieces of bread (they were presented simultaneously) were taken at one time. Tests of the extent of the visual field failed to show contraction or other defects. Subsequent examinations did not reveal further deviations from the normal, except that the animal became stupid and refused to eat, nine days after the operation. At this time he was killed. At the autopsy and subsequent microscopical examinations it was found that the lateral aspects of both occipital lobes were almost completely destroyed. The lesion involved almost all of the cortex in this region, but the calcarine fissures (also visuo-sensory type of cortex) were not affected except at their most posterior extremities.

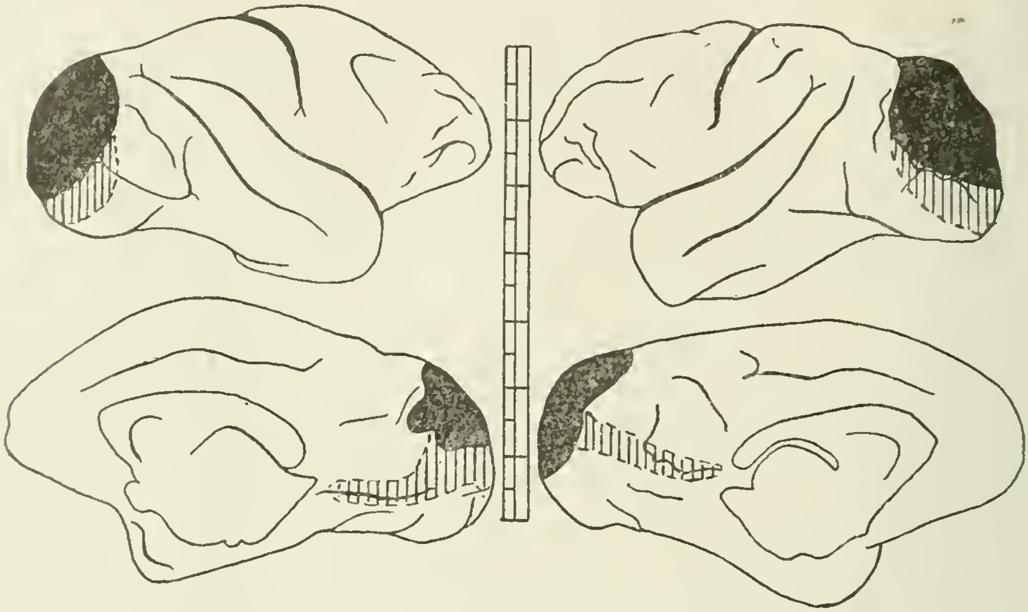

FIGURE 1.—Lateral and mesial aspects of the cerebral hemispheres of Monkey 1, showing the parts of the cortex destroyed by the second operation. The latter is reconstructed from the microscopical examination. Slightly reduced (*cf.* scale).

The accompanying figure (Fig. 1) indicates the extent of the lesions in this animal, the parts in black being the parts which the microscopical examinations showed to be destroyed. For comparison with this, the part of the visuo-sensory cortex which was not destroyed is illustrated by horizontal lines. It will be noted that only a small portion of the visuo-sensory cortex on the convexity of both hemispheres has escaped destruction, but that on the mesial surfaces very little of the calcarine region has been affected.

Monkey 5. — In the training series, this animal was presented with blue (sweet), yellow (bitter), and green (bitter) breads. The discrimination was perfected and operation was performed August 26, 1910. At the operation the cortex was cauterized, the cautery being inserted along the mesial aspect more than in Monkey 1. Four and one-half hours after the operation he appeared fairly normal, picked from the floor pieces of food, the color of which was similar to that of the floor. The movements were fairly accurate when the food was placed not more than 30 cm. from the animal, but inaccurate when it was placed farther away. With the exception of the inaccuracy the monkey was quite like a normal animal for several days, but then developed visual disturbances. A test of the visual fields was made by placing fruit on the ends of wires and having these approach the animal from all quarters. It was found that the food held in the upper left field was always seized immediately, but that in other parts it was not as quickly or as accurately taken. Tests with the colored breads for determining the retention of the color discrimination ability were rather unsuccessful at first, but later tests showed that the animal retained this ability to some extent, but the tests could not be counted entirely successful. On the succeeding days the animal showed greater inaccuracies. Seven days after the operation food was obtained by the animal only by groping on the floor and by taking the food which was placed within a few centimetres of its nose. This remark does not apply to food held in the upper left field, since the animal appeared to be able to sense food in that direction although the movements which were made were rather awkward. On the tenth and eleventh days tests showed the animal to be practically blind.

The animal was killed and the autopsy performed on the eleventh day. The results of the microscopical examination are shown in the accompanying figure (Fig. 2), which indicates that the greatest amount of destruction has taken place along the calcarine fissure, especially on the left side. On this side it will be noted that the cortex surrounding the calcarine fissure has been practically completely destroyed, while on the other side the destruction around the same fissure is limited to the posterior portion. The comparison of these figures with those of Monkey 1 shows that these lesions are somewhat complementary in extent, *viz.*, that the lesion in Monkey 5 involved more of the visuo-sensory cortex surrounding the calcarine fissure, while that in Monkey 1 was chiefly of the visuo-sensory cortex on the lateral portions of the cerebrum. Although the histological pictures are not entirely clear, it seemed likely that the primary lesion on the left side was similar to that on the right, and that the involvement of the anterior portion of the calcarine fissure was a secondary extension of the lesion.

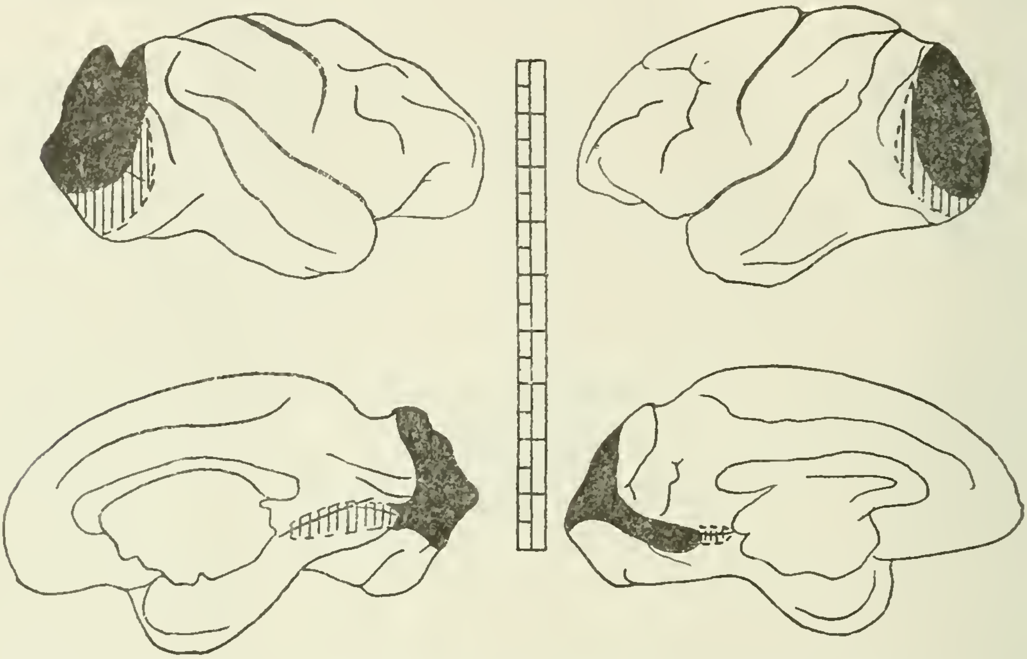

FIGURE 2. — Lateral and mesial aspects of the cerebral hemispheres of Monkey 5, showing the extents of the lesions produced at the operation. Slightly reduced (*cf.* scale).

The other six monkeys were operated upon in such a way that the lateral portion of the visuo-sensory cortex was the seat of the greatest destruction, and in all of these animals the lesions corresponded closely with those in Monkey 1 and the defects accompanying the operation were similar to those in this animal.

The observations which were made on all animals show that destruction of the lateral portions of the so-called visuo-sensory cortex is accompanied by derangements of a character which are not strictly visual. The results of the destruction of these regions are not as marked as those which have been reported by many previous investigators, and it will be necessary to consider what the various facts are and how they may be explained.

The effects of the operations may be grouped into four general classes: (*a*) the ability of the eyes to move; (*b*) the extent of the visual field; (*c*) the visual discrimination ability and (*d*) the ability of visuo-motor co-ordination. In none of the animals were all of these factors affected by the operation, and it will be necessary to refer to animals other than those which have been described here.

Pupillary disturbances were found in three animals (Monkeys 1, 2, and 8). One animal (Monkey 2) showed tremor of the eyelids; another animal (Monkey 1) showed an inward and upward turning of the eyes, and in Monkey 8 the eyes were directed toward the right and this was accompanied by a nystagmus. A fourth animal, at the time the occipitals were being cut, exhibited a slow upward rotation of one eye. Since these oculo-motor disturbances were found in only four of the seven animals, it cannot be concluded that the effect or effects of the operation were principally visuo-motor. This is also borne out by the fact that the defects which were noted by me lasted for only a few hours or days, and it appears probable that these are irritative phenomena rather than phenomena of defect. In all animals eye movements, *e. g.*, those of following a moving piece of food, were normal.

The tests of the extent of the visual field were not successful in all animals, but the observations indicate no correlation between any particular portion of the field and the lateral part of the visuo-sensory cortex.

It may be said that with the exception of Monkey 5 the operations did not produce any alteration in the ability of the animals to discriminate objects. In all cases the animal was able to pick objects from the floor of the cage, to select objects and to convey them to the mouth. Monkey 5, as has been noted, showed an inability to do this after some days. All the animals retained the ability of color discrimination, and all showed evidence of ability to discriminate different kinds of food. Before the operations some of these animals had exhibited distinct preferences for certain kinds of food, and these preferences were shown after the operation by the selection of these foods and the disregard of others. In these experiments foods were placed at such a distance from the animal and often so close together that it would stretch a point to conclude that discrimination could have taken place because of differences in the odor of the foods, for we should have to conclude that foods only 2 to 3 cm. apart could be detected by the direction of the individual odors.

No paralyses were noticed, but the movement disturbances (grasping of food) were similar in all animals. The variations were of two kinds, time and accuracy. All of the animals showed one or the other, and usually both, of these variations from the normal. In the case histories of each of the experiments it was repeatedly noted that the movements were deliberate, or were inaccurate, or were slow, or were indecisive. The movements of the hand in the grasping of food and other objects were inaccurate, however, only when the eyes were a factor in the production of the movement. It was repeatedly noted that when food and other objects were held so that they touched the hair of the animal the food was accurately grasped and conveyed to the mouth. Similarly, when food was held in front of the nose, so that it could be smelled, the movements were quick and accurate. It is apparent that the defect present in these cases is a disturbance of association between the visual sensations and the movement. We know that simple movements of this character depend upon a number of factors, by the proper combination of which we get accuracy and speed. If any of the factors be disturbed, either the speed or the accuracy, or both, may be impaired. We may divide these factors into two different kinds, motor and sensory. If one of these be disturbed, movements as a whole may be affected or may be lacking. For example, if the arm be paralyzed the movement is impossible, even though visual discrimination takes place. If, on the other hand, an animal be blind, the animal cannot grasp food because it cannot see it. It makes no difference in a general way which of these two primary elements be affected, the result is eventually the same.

That the results which were obtained in these animals were not due to a motor defect is clearly evidenced by the fact that when the animal made grasping movements for objects held close to the body, these movements were performed, and repeatedly performed, with great accuracy. Only movements connected with visual processes were inaccurate and slow. Considering the sensory side it was noted that all of the animals were able to discriminate one kind of food from another and one color from another. This indicates very plainly that the visual discrimination function, *per se*, was not affected, and this could not have produced the disturbances of movement. We must, therefore, look farther for an explanation of the phenomena.

We know that in the production of any movement elements other than the visual play a part. These elements, which we must consider in any visuo-motor association, are the sensory elements connected with the movements of the eye. There are two of these: the adaptation of the eye (movements of the lens in the formation of the image) and the accommodation of the eye (action of the extrinsic eye muscles). If we rule out the possibility of defect of the visual sensations, and of paralysis, as we have done, we are forced to conclude that the inaccuracies in movement associated with the lesions of the lateral portions of the occipital cortex, are due to some kind of disturbances of the sensations from the eye muscles. From these experiments we are unable to determine which of the two factors is the more important in the production of the abnormality, and it seems unlikely that a further attempt at such a differentiation will be successfully made on account of the limitation of method. The lack of the means of communication by speech limits our inquiry, and the less direct methods give equivocal data.

SUMMARY.

The results of this study show that upon extirpation of the lateral portions of the so-called visuo-sensory cortex, defects are produced which are not visual in character, but which may be considered visuo-motor. These defects do not depend upon motor inability nor upon visual defects *per se*, but upon changes in the sensory or afferent elements of the portions of the eyes which are normally used in the production of oculo-motor associations. It appears to me very probable, therefore, that the parts of the brain which have been destroyed have a sensory function, but this sensory function is one connected with the eye muscles and not necessarily with the retina.

THE PHYSIOLOGICAL EFFECTS OF ALKALOIDS OF ZYGADENUS INTERMEDIUS.

By PHILIP H. MITCHELL and GEORGE SMITH.

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ZYGADENUS intermedius is one of the several varieties of the plants formerly called *Zygadenus venosus* and popularly known as death camas. They have also been designated by a number of other names, among which are wild onion, mystery grass, lobelia, and poison sego lily. Their economic significance, as pointed out by Chestnut and Wilcox,[1] is considerable because of their prevalence on the sheep and cattle ranges of many western states and their highly toxic effect on the animals, especially sheep, which eat them. Starting up in the spring earlier than the grass but resembling it in form and texture, these plants are so attractive to sheep that they are an enemy which the rancher must reckon with. During the season of 1900 in Montana alone 3,030 sheep were reported to be poisoned by various varieties of *Zygadenus*.

Chestnut and Wilcox[1] have carried out some experiments on the injection of watery and alcoholic extracts of *Zygadenus venosus* as well as feeding experiments. They considered that some, at least, of the plants which they used may have been *Zygadenus intermedius*. The sheep and rabbits used in their experiments showed symptoms of poisoning, such as dizzy movements, slow and labored or convulsive respiration, and finally a deep coma which, if sufficient poison had been administered, terminated fatally. The effects were apparently the same after injection of extracts or feeding of plants, and were identical with those observed in animals poisoned on the open ranges. These authors showed that all parts of the plant contain toxic substances, so that the belief formerly held that animals must eat the bulb or root to be poisoned is erroneous. They found that of the substances which they tried as antidotes potassium permanganate was the most effective. For sheep and cattle they advised giving it in the form of a drench consisting of aluminium sulphate as well as potassium permanganate dissolved in water.

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[1] Chestnut and Wilcox: U. S. Department of Agriculture Bulletin No. 26, 1901.

No further work on the effects of this poison has, so far as the authors know, been reported. Little is known of the chemical nature of the active principle. Slade [2] concluded from certain color tests that the plants contained the alkaloids sabadine, sabadinine, and veratralbine. Heyl and Raiford [3] have made alkaloidal preparations from leaves, flowers, bulbs, and roots of the *intermedius* variety and have shown that alkaloids are especially abundant in the flowers.

Some alkaloidal material prepared by them was sent to the authors of the present paper for the purpose of determining its toxicity. The results of injections into guinea-pigs were sufficiently complex and interesting to warrant a more detailed analysis of the pharmacological action of the preparation. This report is concerned with experiments carried out with that preparation only. It consisted of alkaloids, presumably a mixture, prepared from various parts of *Zygadenus intermedius* according to the methods reported by Heyl and Raiford [3] and held in 17.5 per cent aqueous solution in the form of sulphates.

EXPERIMENTS.

I. **The fatal dose for guinea-pigs.** — To obtain some idea of the relative toxicity of the preparation, intraperitoneal administration to guinea-pigs was employed. Injections of the alkaloidal solution diluted with sterilized physiological saline were made aseptically.

In Table I the results are shown arranged in the order of the dose per 100 gm. of guinea-pig. The fatal dose is seen to be between 4.6 and 5.1 mgm. per 100 gm. of animal. It is probably nearer the lower than the upper of these limits, because the animal which received 4.6 mgm. per 100 gm. recovered only with great difficulty after a long period, eighteen hours, and seemed at least twice during that time to be in its death struggle. This injection, therefore, was probably very near the fatal dose. In the case of the injection of 5.1 mgm. per 100 gm., on the other hand, death ensued in so short a time, twenty-five minutes, that this dose was perhaps a little more than the fatal one. These results indicate therefore that we have a substance of marked toxicity.

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[2] SLADE: American journal of pharmacology, 1905, lxxvii, p. 262.

[3] HEYL and RAIFORD: Journal of the American Chemical Society, 1911, xxxiii, p. 206.

TABLE I.

INTRAPERITONEAL INJECTIONS INTO GUINEA-PIGS.

Weight of guinea-pig.	Volume of solution.	Amount of sulphate.	Dose per 100 gm. of animal.	Result.	Time between injection and death.	Time between injection and recovery.
gm.	c.c.	gm.			min.	hours
485	2.0	0.05	0.0111	Death	19	..
364	1.6	0.04	0.0104	Death	23	..
315	4.0	0.028	0.0090	Death	21	..
322	3.5	0.0245	0.0076	Death	31	..
332	2.9	0.02	0.0060	Death	26	..
290	2.1	0.015	0.0051	Death	25	..
305	2.0	0.014	0.0046	Recovery	..	18
300	1.0	0.0035	0.0011	Recovery	..	3

It seemed important also to test the effect of administration *per os*. The undiluted alkaloidal solution as furnished us was put in small gelatin capsules and fed to guinea-pigs by forcing the capsules into the back of the mouth through a glass tube. The results are arranged in Table II, and apparently indicate that a comparatively large quantity (0.2 gm.) is required for the fatal effect.

The actual fatal dose absorbed from the digestive organs is not, however, as large as these figures would seem to show. A considerable quantity of the material fed was vomited up before it was absorbed, because the substance, as will be explained below, is a very powerful emetic. Another factor tending to diminish the toxicity when fed is the power of the organism to effect an oxidative destruction of the alkaloid before a fatal amount has been absorbed. This effect is illustrated by the rather quick recovery of guinea-pigs from the effects of injection of small doses. For example, in the last experiment recorded in Table I, 3.5 mgm. produced very profound symptoms of poisoning, but these had entirely passed away within three hours. In another experiment, not recorded above, the subcutaneous injection of 3.7 mgm. produced undoubted effects which had all disappeared in an hour and a half. It does not seem necessary then to conclude from these experiments that gastro-intestinal digestion destroys the alkaloid or diminishes its toxicity, but it seems reasonable to conclude that the tendency to vomiting, the slowness of absorption, and the rate of destruction of the alkaloid account for the relatively large quantity required to kill when fed.

TABLE II.

PER OS ADMINISTRATION TO GUINEA-PIGS.

Weight of guinea-pig.	No. of capsules used.	Amount of sulphate.	Dose per 100 gm. of animal.	Result.	Time between feeding and death.	Time between feeding and apparent recovery.
gm.		gm.				min.
334	1	0.0175	0.0053	Recovery	..	30
352	4[1]	0.140	0.040	Recovery[2]	..	About 12 hours
278	6	0.21	0.0755	Death[2]	About 12 hours	..

[1] Last two capsules given one hour after the first two.

[2] Occurred during the night and was not exactly known.

II. **The effects on guinea-pigs.** — After subcutaneous or intraperitoneal injection or after feeding, the alkaloid produces the same symptoms of poisoning. There are marked insalivation and frequently repeated vomiting, which begin very soon after the substance is administered and persist nearly as long as any symptoms can be observed. During the first few minutes of the onset of the effects the animal jumps and runs about the cage excitedly in the intervals between vomiting. It soon begins, however, to lose control of its muscles. The hind legs are usually affected first, but finally all the limbs become useless and the guinea-pig lies on its side completely prostrated. The respiration, at first rapid, has by this time become slower than normal and very labored. It is frequently interrupted by short periods of very convulsive breathing. Although quite unable to make any co-ordinate movements, the animal is exceedingly irritable and responsive to reflex stimuli. The lightest touch will start a struggle, and as the symptom complex advances there comes a stage when the struggles become spasmodic and later typical tetanic spasms occur. The guinea-pig now behaves quite like an animal poisoned with strychnine, and at the slightest irritation, such as a jar of the cage or a breath of wind, exhibits profound tetanus. All of its muscles are rigid and the body stiffened out in an extended position sometimes for ten seconds at a time. The heart rate as detected by allowing the guinea-pig to lie in the observer's hand so that the cardiac impulse may be felt is found to be much slower than normal, and this condition persists until recovery is quite far advanced or death occurs. Defecation takes place at frequent intervals throughout the development of effects. Micturition is also generally observed. If the fatal dose has been given by injection, death occurs after an interval of about twenty to thirty minutes. No relationship between the dosage administered intraperitoneally and the time required to kill is to be seen in our results. Two typical protocols follow:

Experiment No. 1. *Jan.* 30, 1911. — Female guinea-pig; weight, 845 gm. Dose: 0.05 gm.; volume, 2 c.c.

3.25 P. M. Intraperitoneal injection.

3.27 P. M. Vomiting; claws mouth.

3.29 P. M. Spasmodic twitching; retching; jumping; gagging; squealing.

3.30 P. M. Defecation; vomiting; lies on side. Cannot right itself.

3.32 P. M. Respiration very irregular.

3.33 P. M. Short, recurring tetanic spasms.

3.35 P. M. Respiration shallow and infrequent; spasms stop. Trembling.

3.36 P. M. Perfectly limp.

3.38 P. M. Infrequent, spasmodic respiration.

3.40 P. M. Muscular spasms.

3.41 P. M. Respiration, 17 per minute.

3.44 P. M. Lid reflex absent.

3.45 P. M. Tetanus.

3.46 P. M. Dead. Heart in complete diastole. Peristalsis of the intestines very marked.

Experiment No. 2. *Jan.* 30, 1911. — Male guinea-pig; weight, 352 gm. Dose: 0.0037 gm.; volume, 1.5 c.c.

4.06 P. M. Subcutaneous injection.

4.09 P. M. Insalivated.

4.11 P. M. Vomits a little.

4.13 P. M. Vomits repeatedly.

4.39 P. M. Labored respiration interrupted by rapid respiration. Spasm.

4.40 P. M. Control of limbs is lost. Labored and slow breathing.

4.41 P. M. Respiration, 49–50 per minute.

4.42 P. M. Slight spasm.

4.44 P. M. Respiration very irregular; heart fast. Spasm.

4.46 P. M. Respiration, 24 per minute; sometimes nine seconds elapse between breaths. Tetanic spasms; heart rate, 81 per minute.

4.49 P. M. Respiration, 23 per minute; still tries to right itself although unable to control limbs.

4.52 P. M. Spasms.

4.53 P. M. Tetanus in back muscles when touched; very bad spasms.

4.58 P. M. Heart rate, 42 per minute.

5.00 P. M. Respiration, 29 per minute; lid reflex present.

5.02 P. M. Very acute spasms; tetanus continues for three minutes without stopping.

5.11 P. M. Acute spasms; lid reflex present.

5.14 P. M. Spasms; great respiratory distress.

5.17 P. M. Hyperpnœa alternating with apnœa.

5.21 P. M. Spasms; tetanus; defecation.

5.29 P. M. Heart rate, 48 per minute.

5.32 P. M. Spasms; defecation; much urine passed.

5.33 P. M. Heart very slow.

5.35 P. M. Spasms; eyes closed. Reflex present.

5.39 P. M. Respiration, 32 per minute.

5.42 P. M. Spasms; respiration, 46 per minute.

5.54 P. M. Respiration, 23 per minute.

5.55 P. M. Acute spasms; defecation.

5.58 P. M Spasms; tries to gain feet.

6.01 P. M. Copious flow of urine.

6.12 P. M. Acute spasms; spasms now follow slightest stimulus.

6.30 P. M. Regained control of fore limbs. Spasms no longer follow stimuli.

6.57 P. M. Labored and spasmodic respiration.

6.59 P. M. Respiration easier.
7.01 P. M. Heart beat, 100 per minute.
7.10 P. M. Vomits again. Appears nearly recovered.
Jan. 31, 1911. — 9.00 A. M. Entirely recovered.

III. **The effects on frogs.** — The effects of administration to frogs were not very striking. It was thought that because of the tetanic spasms in guinea-pigs this poison might affect the spinal cord somewhat as strychnine does. Experiments with frogs did not fulfil that expectation. Two injections into the dorsal lymph sac of frogs with the brain pithed in which 0.175 gm. of material was administered in one case and twice that amount in the other caused some slight irritability, but the animal soon became entirely limp and unresponsive to stimuli. To another frog about 0.1 gm. was injected without pithing any part of the nervous system. Some twitchings and spasmodic movements occurred, but in a few minutes the animal seemed paralyzed, gave no further response to stimuli, and soon died. In all three frogs there was a noticeable reddening of the skin, and the blood vessels in the web of the foot seen under the microscope appeared to be dilated.

IV. **The effects on dogs.** — To more accurately trace the action of the alkaloidal preparation, intravenous injection into dogs was employed. The animals, seven in all, were anæsthetized in each case with ether followed by A-C-E- mixture. The alkaloidal material diluted with physiological salt solution was injected into the right femoral vein, while a tracing of blood pressure in the left carotid artery was recorded by the aid of a mercury manometer. A record of respiratory movements made by using a simple cord and pulley device to connect a recording lever with a skin suture in the thoracic or abdominal wall of the dog was simultaneously obtained.

The first injection invariably caused a fall in the blood pressure within a few seconds. Its amount was roughly proportional to the amount of material injected. 0.0035 gm. of the alkaloidal sulphate given in 1 c.c. of a fifty-fold dilution of the original preparation to a dog of 13 kilos caused a fall of blood pressure equal to 41.6 mm. of mercury, while the injection of 0.0105 gm. in 5 c.c. of the same dilution in a dog of 20 kilos produced a fall of 82 mm. The proportionality, however, was not always maintained. The quickness of the recovering rise also depended on the amount injected, and if more than 10 mgm. of the alkaloidal sulphate was given the recovery was very slow. In no case did the blood pressure ever return to quite as high a level as that recorded before the first injection. The cause of this depressor effect is not a simple one. The most potent factor in the initial drop is the marked slowing of the heart rate. That this is due for the most part to an effect on the cardio-inhibitory centre appears from the fact that after section of both vagi an injection of the quantity usually employed did not cause the usual striking depression of the heart rate but only a very slight decrease. A second factor involved in the fall of blood pressure is vaso-dilation. Even when the pulse had quite recovered to its initial rate as seen in several tracings, the blood pressure was found to be still low, and only recovering so slowly that after some half-hour's observation it did not reach its original level. Also, the arterial pressure fell too much when injections were given after double vagotomy to be accounted for apparently by the slight slowing of the heart.

The effect on respiration is, in general, to slow it by a prolongation of the expiratory phase. This effect is slightly modified after the vagi have been severed.

A tracing to show the effect of injection without previous vagotomy is given (Fig. 1). The tracing seems to show that the alkaloid acts on the cardio-inhibitory and respiratory centres, or at least produces effects which involve them.

The results so far discussed are those obtained by an initial injection of small quantities of the alkaloid. Subsequent injections or the administration of larger quantities produced more complex effects. Second, third, and fourth injections of quantities comparable to that ordinarily used for our first, produced a successively smaller effect in decreasing the already lowered blood pressure. After a considerable quantity, for example, 0.0665 gm. had been given, a compensatory hastening of the heart rate produced a slight rise in blood pressure, and this effect was obtained after section of the vagi with comparatively small quantities of the alkaloid. Fig. 2 shows the effect of injection of 0.0105 gm. into a dog of 8.2 kilos after a previous injection, about twenty minutes earlier, of the same quantity. The rise in blood pressure amounts to 36 mm. of mercury, and the change in the heart rate is from 117 per minute before the injection to 157 per minute shortly after. In this case the vagus nerves were severed at the beginning of the experiment and the first injection gave a slight fall in pressure.

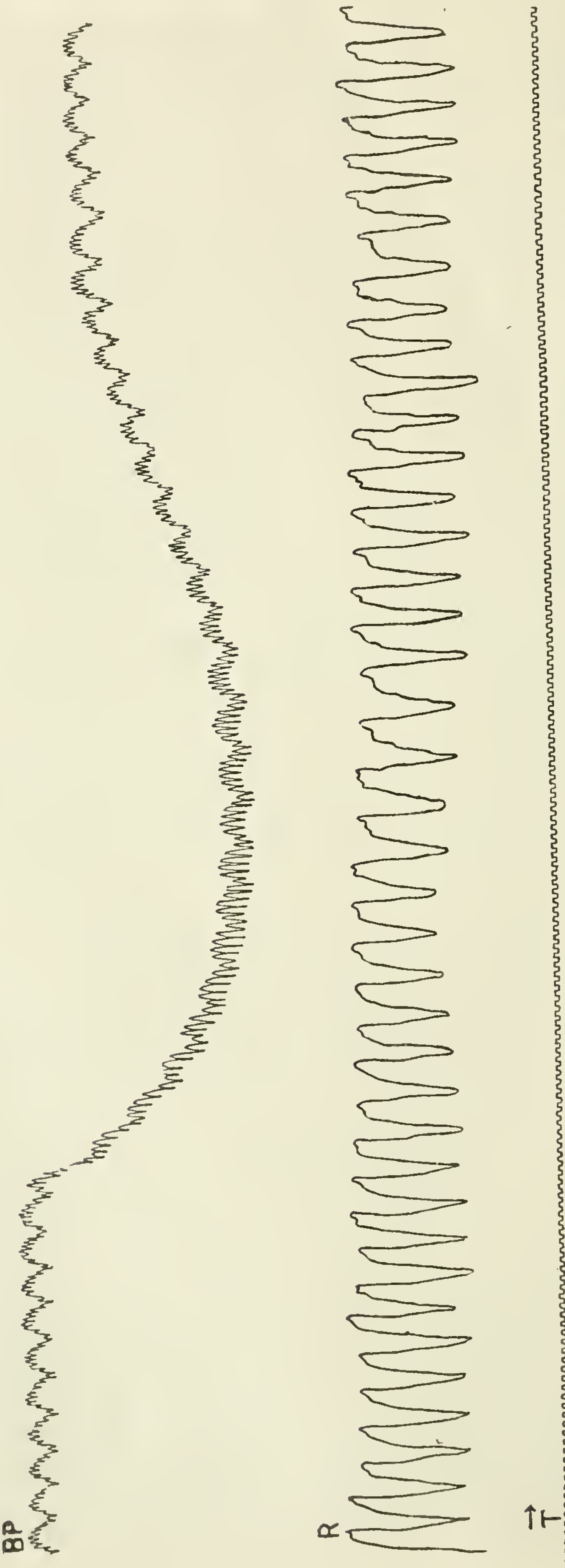

FIGURE 1. — Effect of first injection of small quantity with vagi intact. Upper tracing shows carotid pressure, lower one respiration. Time = 0.6 sec. At *A* 0.0035 gm. of alkaloid in 1 c.c. of saline was injected. Dog weighed 22 kilos.

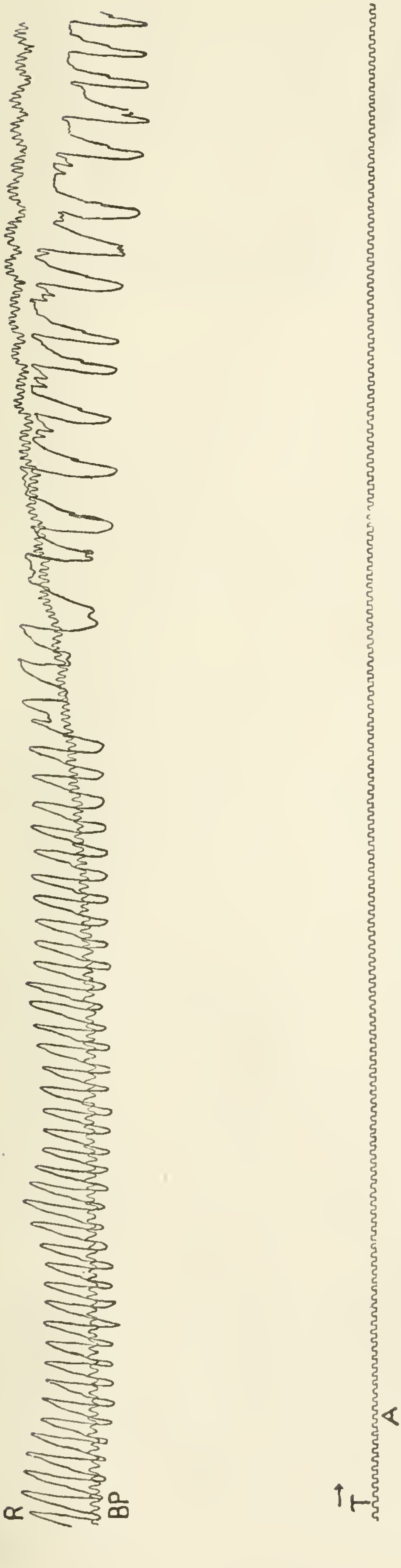

FIGURE 2. — Effect of a second injection, after section of the vagi. Tracing of carotid pressure partly superimposed on respiration curve because the pressure was lowered from the effects of the first injection. Time = 0.5 sec. At *A* 0.0175 gm. of alkaloid in 5 c.c. of saline was injected.

When sufficiently large quantities of the alkaloid had been administered to a dog, its heart showed an effect distinctly different from that produced by the previous injection of small quantities. Instead of the decrease of the heart rate with increase in the force of the beat seen in the initial injections or the increase in rate with slight decrease in force observed after further injections, there appeared in the more advanced stages of poisoning a marked fluttering of the heart. A tracing of such an effect is shown in Fig. 3. Sometimes the heart action as shown by the blood pressure curve would produce a series of very irregular beats interrupted at quite regular intervals by groups of three or four regular but very quick, shallow beats.

The effects of larger quantities of the poison on respiration were also noteworthy. Instead of the mere slowing of respiration, there appeared a considerable irregularity characterized by movements more or less convulsive. This effect in a mild degree can be seen in Fig. 2.

One of the most interesting effects of this substance was that on intestinal peristalsis. Even the smallest dose administered, 0.0035 gm. given intravenously to a dog of 20 kilos, caused in a few minutes unmistakable intestinal rumblings. In all the dogs used defecation occurred several times, even though stools had previously been passed at the time of etherizing. In the course of an hour, after three or more injections of small quantities, fluid or semi-fluid feces were

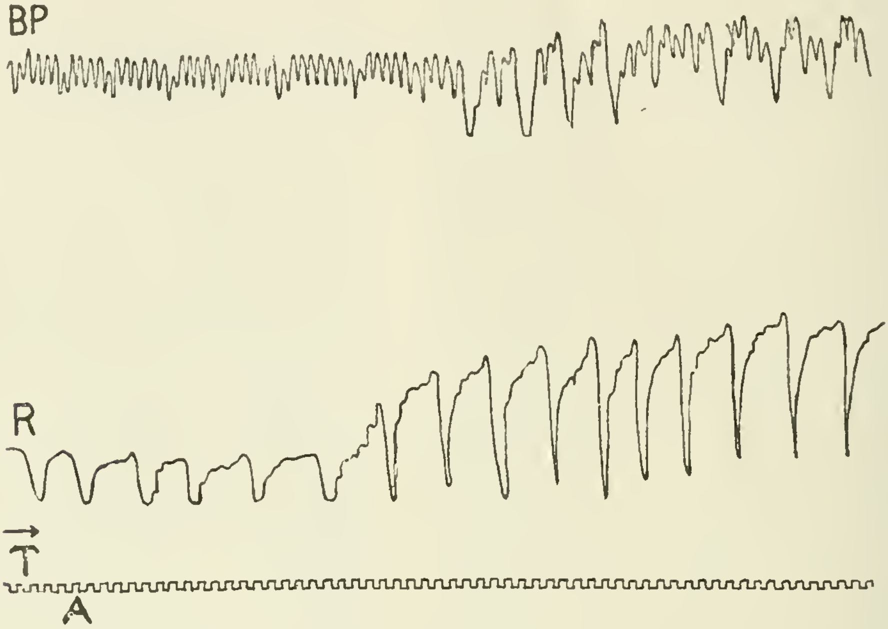

Figure 3. — The effect on the heart of cumulative action of the alkaloid. Upper tracing shows carotid pressure, lower one respiration. Time = 0.6 sec. At *A* 0.0175 gm. of the alkaloid in 5 c.c. of saline was injected. The vagi were intact. By means of three previous injections, 0.0315 gm. of the substance had been given.

observed in each case. Micturition also took place in many of the dogs during the experiment. This involuntary evacuation of intestines and bladder in anæsthetized dogs was plainly the result of the action of the alkaloid, because effects followed quickly after injection and in the case of defecation were proportional to the amount given. Such results confirm our observations with guinea-pigs. The influence of the substance on the vomiting mechanism of dogs was not tested, as in guinea-pigs, because no injections were made without previous anæsthesia.

Several of the dogs while still anæsthetized were killed by intravenous injection of a fatal dose. In each case the heart beat became very irregular and soon stopped. Invariably the heart failed before respiration. At the moment respiration ceased, however, the dog passed into a death struggle exhibiting tetanic spasms of the entire body. The convulsion was always brief, and though in two cases followed by a few spasmodic respiratory movements was never, so far as we observed, succeeded by any revival of the heart beat. The heart was found in the opened thorax in complete diastole, as was seen also in post mortem examination of the guinea-pigs.

No attempt to determine the fatal dose for dogs was made. The smallest quantity which gave a fatal result was 0.105 gm. in 3 c.c. of saline injected intravenously into a dog of 10 kilos without previous injections.

Our experiments have led to the following conclusions:

1. The alkaloidal preparation from *Zygademus intermedius* slows the heart rate by action apparently on the cardio-inhibitory centre.
2. It slows respiration by an effect involving the respiratory centre.
3. It causes vaso dilation.
4. In quantities approaching the fatal dose it hastens the heart rate and produces both irregularity of the heart beat and convulsive respiration.
5. The fatal dose given intravenously to dogs stops the heart before respiration ceases.
6. The fatal dose for guinea-pigs is between 4.6 and 5.1 mgm. per 100 gm. of animal.
7. It has a very powerful action, whether injected or fed, both as a purgative and an emetic.

The authors wish to acknowledge with thanks the assistance of Mr. H. A. Swaffield, who aided in some of the experiments.

RHYTHMICITY IN THE TURTLE'S HEART AND COMPARISON OF ACTION OF THE TWO VAGUS NERVES.

By WALTER E. GARREY.

[*From the Physiological Laboratory of Washington University, St. Louis.*]

THE difference in the action of the two vagi of the turtle is a fact so striking that it could hardly have escaped the notice of any one working in this field of physiology; the right vagus is so much more effective as the inhibitory nerve. There is, however, a marked variability in the quantitative differences which one finds not only in different species but also in the individuals of a given species. It was found by A. B. Meyer,[1] that the left vagus failed completely to stop the heart in the eight specimens of Emys lutaria, which he examined, and Gaskell[2] makes a similar observation on Testudo græca. The phenomenon has been more carefully studied on the slider terrapin (Pseudemys rugosa) by T. Wesley Mills,[3] and has been recorded on other species by Kazem-Beck[4] and Gueynot.[5]

I have studied the same phenomenon in four common species of turtle (Pseudemys rugosa, Chrysemys marginata, Chelydra serpentina and Terepene sp?) and have found distinct differences in the action of the two vagi in all of them. The character of these differences will appear in the description of my experiments.

In spite of the frequency with which the observation has been made, no advance has been made in the explanation of the differences beyond Meyer's view that "the nerve fibres supplying those muscular fibres of the sinus which by their automatic contraction originate the rhythmical beats of the heart, run almost exclusively in the right vagus nerve."[6]

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[1] MEYER: Hemmungsnervensystem, Berlin, 1869, ref. GASKELL, *cf. infra.*

[2] GASKELL: Journal of physiology, 1883, iv, p. 82.

[3] MILLS: Journal of physiology, 1885, vi, p. 247.

[4] KAZEM-BECK: Archiv für Anatomie und Physiologie, 1888, pp. 325–352.

[5] GUEYNOT: Comptes rendus de la Société de Biologie, 1907, pp. 1025, 1032, 1145, 1190.

This statement in reality contains the germinal idea which underlies the true explanation, *viz.*, that in the turtle's heart different vagus fibres innervate separate parts of the heart, and that those fibres which are ineffective do not affect the rapidly beating part of the heart which acts as the pace-maker. The investigations which are recorded in this paper prove this conception to be the correct one, for it is found that in the turtle there exists a pronounced preponderant inhibitory action of each vagus upon the corresponding half of the heart. This homolateral action is most pronounced at the base of the heart and to a lesser extent is to be observed upon the auricles as well. It will be shown that in those cases in which the left vagus fails to affect the rhythm it may still be able to affect very profoundly certain other regions of the heart, it will be further shown that the left vagus may inhibit completely the auricles and ventricle, but that the contralateral (right) basal portion of the heart (right caval veins) may still beat in their normal rhythm. The preponderant action of the right vagus, it will be shown, is due to the fact that its action is directly upon those basal parts of the heart which initiate the rhythm, *viz.*, the right caval veins. An alteration of rhythm or stoppage of the ventricle is no criterion for the action of the vagi; the effects upon each part of the heart must be studied before any adequate conception of the actual effectiveness of the vagi can be ascertained.

THE POINT OF ORIGIN OF THE BEAT, AND THE RHYTHMICITY OF THE VEINS AT THE BASE OF THE TURTLE'S HEART.

If we are to learn why the right vagus affects the heart rhythm more profoundly than the left, we must first learn the portion of the heart which acts as the pace-maker and must show that the preponderant action of the effective vagus is really on this portion of the heart. This necessitates a careful examination of the region of the sinus and great veins which open into it. This portion of the heart has been much neglected by investigators. Most careful work in this direction has been done by MacWilliam [7] on the heart of the eel, in which he states that the beats originate simultaneously on either side of the inter-jugular part of the sinus, *i. e.*, at the ostia of the jugular veins; and that the sinus contracts subsequently. Engelmann,[8] too, has made a careful study of the beat of the veins entering the frog heart. He found that isolated pieces of these veins beat spontaneously, but that in the intact heart these veins with the sinus act as a unit and beat synchronously. In contrast with these findings, the work of MacWilliam, of Herring, and more recently of Erlanger, has shown that in mammals the beat originates in the region of the junction of the superior and inferior venæ cavæ.

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[6] Quoted from GASKELL: *Loc. cit.*, p. 83.

A suspicion, founded on the action of the vagi, that a condition similar to that existing in mammals might also exist in the turtle led me to an examination of this portion of the heart and to a series of experiments, the results of which fully confirmed my belief that the beat originated on the right side — the side of the more active vagus nerve. Simple inspection will reveal the fact that, owing to the arrangement of the veins and the junction with the sinus of the right pre- and post-caval veins and the great vein from the liver, the development of the right basal part of the heart is far in excess of that of the left side. Inspection further shows that the beat arises in a rather diffuse area which includes the area at the junction of the right pre- and post-caval veins and extends toward the sinus and mouth of the veins from the liver. This area is not sharply defined, and not infrequently the right veins throughout their whole extent seem to beat synchronously with it. Next in sequence, after the contraction of this area, the sinus contracts, the wave of contraction passing thence to, and involving, the right vein. In many cases a distinct pause can be seen before the sinus contracts, and another pause before the involvement of the vein of the left side; these pauses are not long and in every instance the vein of the left side contracts before the auricles do. The time relations are shown in Fig. 1, in which the tracings were taken from the veins which empty into the sinus on the right and left side respectively. On the tracing taken from the right vein there is a distinct indication of a physiologic pause between the wave produced by the contraction of the right caval veins (R) and the sinus (S). On the tracing taken from the left caval vein the waves S, L, and A correspond to the time of contraction of the sinus, left vein, and auricles respectively.

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[7] MacWilliam: Journal of physiology, 1884, iv, pp. 197 *et seq.*

[8] Engelmann: Archiv für gesammte Physiologie, 1896, lxv, pp. 115 *et seq.*

These tracings show that the veins entering the sinus must be looked upon as separate cavities of the heart which are physiologically as independent as the sinus or auricles. In thirty-eight specimens examined it was found that the beat started in the right veins and not in the sinus, as is generally stated to be the case. The distinct pause between the beat of the veins and the sinus effectively disposes of the argument that might be advanced, *viz.*, that in these cases, owing to greater development of the right side, the sinus is displaced to that side, and that in reality the beat originates in that structure; this is not the case, the beat starts in the veins of the right side and involves the sinus subsequently. The wave, in passing from right to left, not only impedes the outflow of blood from the left vein as a result of the contraction of, and rise of pressure in, the sinus, but actually causes a regressive flow of blood in the left vein due to the antidromic direction of the contraction wave in that structure.

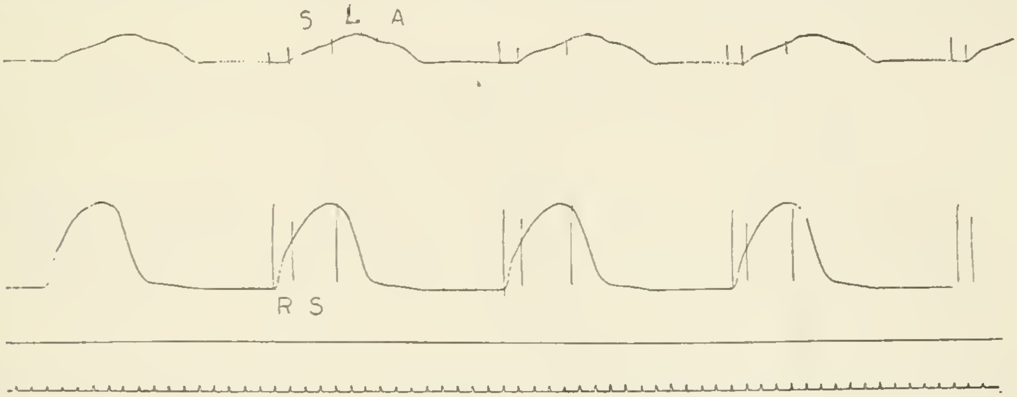

FIGURE 1. — About two thirds the original size. The upper curve is from the left caval vein, the lower from the right caval veins. R = contraction of right veins; S, contraction of sinus; A, contraction of auricles.

Since the rate of the heart is determined by that part which possesses the greatest inherent rate of rhythm, and since the contraction wave likewise starts in the same location, it follows, from what has been said above, that the right caval veins which inherently possess a greater rhythmicity than the other parts of the heart, determine the rate of the whole heart. That this is true may be tested in a variety of ways; some of the methods I have employed will now be considered.

After the veins of both sides have been transsected close to the sinus and the rest of the heart excised, levers may be attached to the cardiac ends of the isolated veins *in situ*, and records made of the relative rates of contraction. Such a tracing is seen in Fig. 2. It is seen that the right veins beat at a rate of 37 per minute and the left veins at the rate of 15 per minute. The ratio of the rhythms is $^{R}/_{L} = 2.5$ approximately.

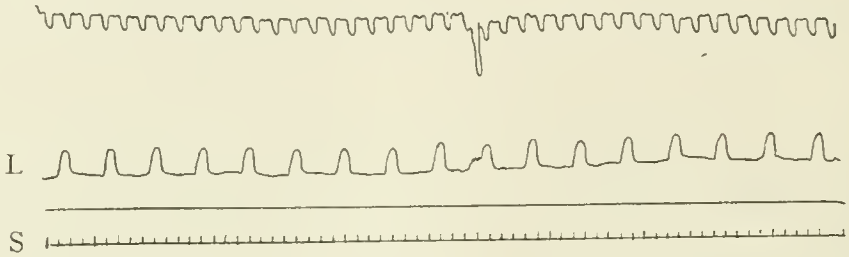

FIGURE 2. — The upper curve is from the right caval veins; a portion of the sinus was left connected with these veins, the contraction of which causes the notch in the curve. L = left caval vein, S = time in seconds.

Another method of determining the point of origin of the rhythm is to cut away, or clamp off different parts of the base of the heart and to note the rate of contraction resulting from the procedure. If the left vein, including a part of the sinus, be separated from the heart, no change in rate is noted. Similarly, if the vein from the liver be in like manner cut away, no change is noted. If, however, the cut be made diagonally in such a way that the exsected piece includes the liver vein, the ostia of the right caval veins and the portion of the sinus between them, the rhythm of the heart at once becomes much slower and the slow rhythm persists and examination shows that the beat now originates, with few exceptions, in the *left* caval vein, involving in the usual sequence the remaining portion of the sinus, auricles, and ventricles. The contraction wave in the left vein is no longer a retrogressive one, but progressive, *i. e.*, toward the heart. The exsected portion which includes that portion of the heart in which the former cardiac rhythm was initiated is found beating at the original rate. Fig. 3 shows the result of such an operation. *A* is a tracing of the normal heart rate; the tracing lever was attached to the ventricle. *B* is a tracing of the same preparation made after the right veins had been tied off; this procedure changed the rate of the heart to approximately one fourth its previous rate, although it was easily seen that the beat still originated at the base of the heart and in fact in the veins of the left side. In *C* the right veins had been cut away, thus eliminating the conspicuous "tug" effects of the right veins. The right vagus is noted to have lost its effect in *B* as a result of the ligature, but the left vagus, which previously had been found to have no chronotropic effect, is now noted to be active; the discussion of the vagus effects will be deferred to another portion of this paper.

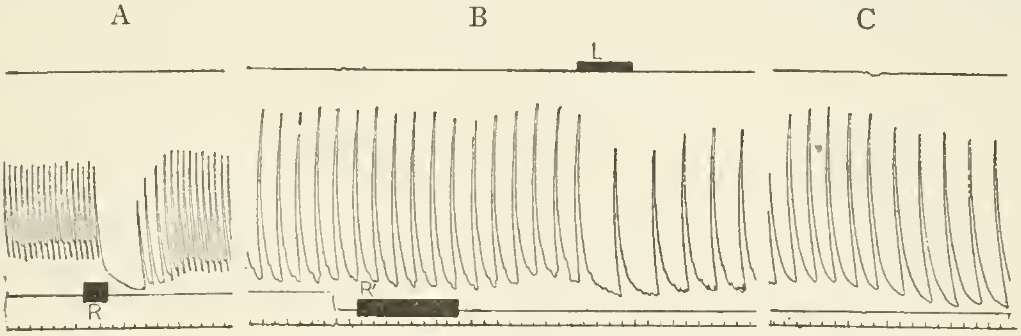

FIGURE 3. — About one half the original size. Tracing by suspension method of ventricular contractions. *A* = normal heart, *B* = after tying off the right caval veins, *C* = after cutting away the right caval veins the tug of the right veins noted in *B* disappears in *C*. The effect of stimulation of the vagi before and after clamping the right veins is shown; at *R* and *R'* the right vagus nerve was stimulated and at *L* the left vagus was stimulated.

In several experiments a transverse cut was made at the sino-auricular junction and auricles and ventricles were removed from the body. The veins of the right and left side with the sinus between them formed a transverse beating strand in which the wave was seen passing from right to left with the original rhythm of the heart. If a median section should be made, we should have (except for the adhering bits of the sinus) the same condition as that in the first experiments considered above (p. 334). In the experiment under consideration, however, the author put a clamp of the Gaskell type in the median line between the two veins, and by the micrometer screw this was tightened till a complete intervenous block existed. In Fig. 4 a tracing is made of such a preparation, and the ratio of the beating veins in this case is seen to be $^{R}/_{L} = 2.1$. The ratio 2.1 is not invariable and is not due to an incomplete block, as a subsequent section demonstrated. The rhythm of the left portion is far less regular than that of the right side, and the independence in the beat is also evident when each vagus is stimulated; the piece on the homologous side only stops beating, and during recovery the chronotropic effects are evident on the same side only; the intervals vary and the beat of the two sides shows no integral or constant ratio.

In another series of experiments a sagittal section was made in the median line through the whole heart, thus dividing it into two approximately equal parts. The immediate effect of this splitting operation is complete inhibition of both the lateral halves. This condition, however, soon wears off and regular beats are inaugurated; these start invariably in the right half before they do in the left half, indicating again the greater rhythmicity of the right side. Later the left side begins to beat, but it always beats in a slower rhythm than the right side. Both time of onset and rate, then, indicate the difference in rhythmicity of the two lateral halves of the basal parts of the heart which initiate these rhythms, *viz.*, the veins, for it is a striking fact that can be easily verified that in the left half of the heart the beat does originate in the left vein, which prior to the section followed in sequence the beat of the right side. The rate for the right half is the rate of the original beat of the heart, that for the left half is always slower.

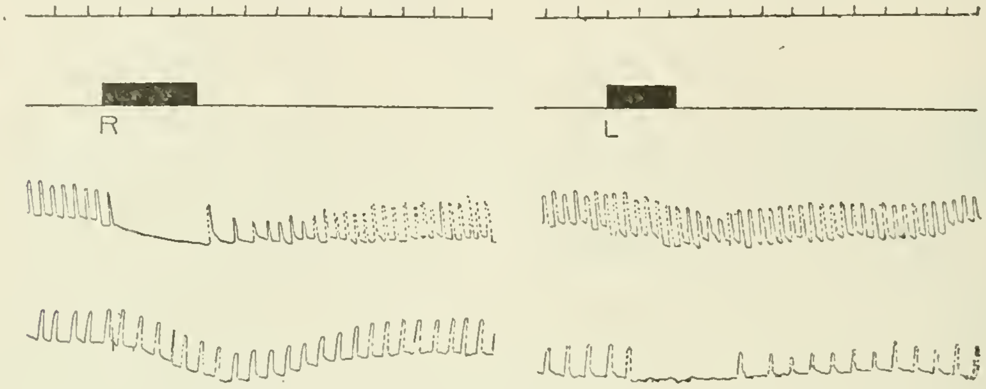

FIGURE 4. — The upper curve is from the right caval veins, the lower from the left caval vein. *R* and *L* show points of stimulation of the right and left vagi respectively. *T* = time in five seconds.

Concerning the question of the ratio of the rate of the right to the left side it should be said that there is no very great constancy in different individuals, but it was found to be as low as two and as high as four. In the earlier experiments of this series higher ratios were obtained, but comparison with the ratios of the isolated veins indicated an error which was revealed upon careful inspection, *viz.*, that veno-sinus and sino-auricular blocks were apt to result from the traumatism of section; some part of the heart less rhythmical than the homologous vein often being responsible for the recorded rhythm, while the vein was found to be beating more rapidly; owing to the block existing in these cases, the beat of the vein did not influence the rate of the heart. These blocks were far less frequent in the right half. The original rhythm of the unmutilated heart was taken as the standard for comparison in all determinations.

It occasionally happened that in the beat of the left half of the "split" preparation the rhythm was initiated in the sinus of that piece without a block existing between the sinus and the left vein. The wave of contraction spread in these cases from the sinus not only toward the auricle, but also in a retrogressive manner onto and up the left vein, indicating that the inherent rhythmicity in these cases was greater for the sinus than for the left vein. This is rarely the case, however, and this variation from the usual may explain the range of $^R/_L$ ratios mentioned above. Although I have repeatedly examined the right half of the split preparation in the same manner, it has never happened that the sinus could beat more rapidly than the right veins. The veins of this side always possess the highest rhythmic power of any part of the heart, and they, not the sinus as is usually supposed, initiate the rhythm of the turtle's heart.

Fig. 5 shows a tracing before (*A*) and after (*B*) splitting the heart sagittally into lateral halves. The rate of the right half is seen to be the same as that of the heart before the operation; — that of the left side is much slower — the ratio of the rates of the two sides, $^R/_L = 3.5$. As was noted above, the rate ratio of the two halves of the split preparation is that of the two right and left caval veins respectively, because they initiate the rhythm for their respective sides.

Whenever a certain ratio between the right and left halves has been established this ratio remains remarkably constant; for example, the following table gives the results of varying the rates of the two halves simultaneously by pouring saline solutions of different temperatures over the heart. The solution, of course, mixed with that already about the heart so that the temperature could not be accurately determined, and is only indicated by letters lest misleading conclusions should be drawn. The temperature of the two halves is approximately the same. The results of this experiment are shown in Table I.

It is thus seen that the ratio of the rhythm of right to that of left side (in this experiment averaging 2.26), remains quite constant, while the actual rates vary from 5.7 per minute to 60 per minute.

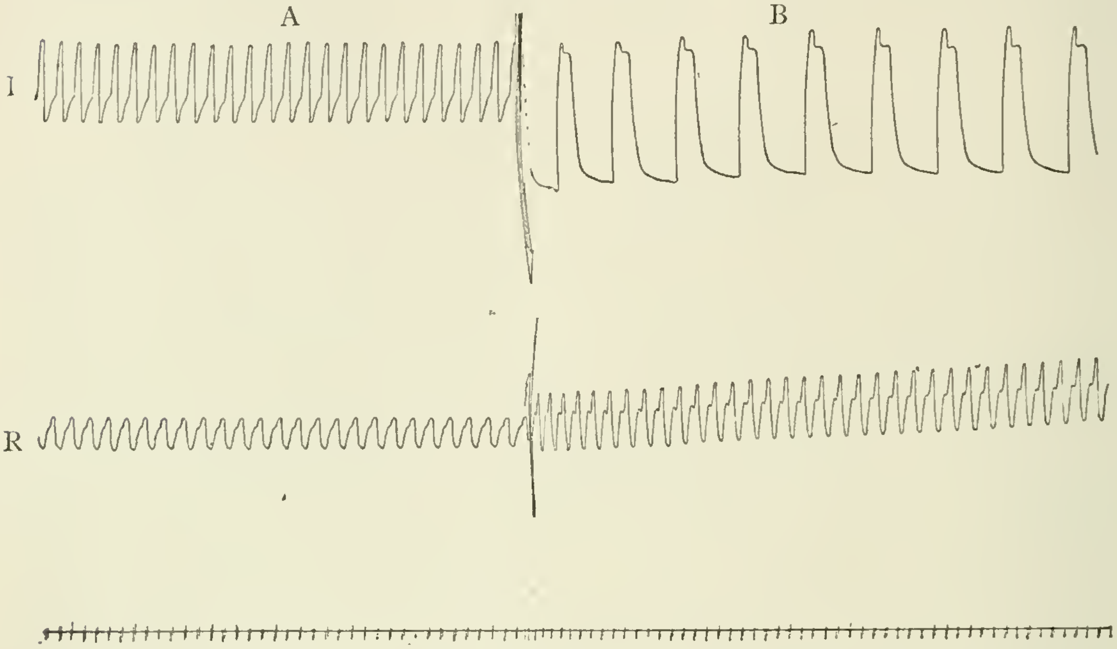

FIGURE 5. — Tracing taken from the right and left auricles *R* and *L* respectively. Between the portions *A* and *B* the heart was split sagittally. The slow rhythm of the left half is thus revealed.

The "split" preparation has adapted itself to another interesting series of experiments which confirm and strengthen the experimental evidence that the two sides of the base of the heart possess different inherent rhythmicity. If the sagittal section be carried in the median plane from the base of the heart toward the apex, but only involving the auricular portion of the ventricle, leaving a good conducting bridge between the right and left ventricular masses thus partially separated, we have a trouser-like preparation which may be likened to the ascidian heart.

This "ascidian" preparation, if it has been successfully made and has no regions of block, will be found to conduct the impulses from the right end to the left. The beat arises in the right pre- and post-caval veins, and the wave involves successively the right part of the sinus, right auricle, right ventricular mass, left ventricular mass, left auricle, left half of the sinus, and left pre-caval vein.

As is well known, the heart of ascidians normally reverses the direction of its beat from time to time, and this is due to the fact that the rhythmicity of the two ends, which initiate the contractions, is practically identical, so that very slight alterations in the conditions existing at either end will determine a reversal — conditions such as temperature or possibly of pressure due to accumulation of blood.

TABLE I.

Temperature.	Absolute rate of right half.	On the left side =	On the right side.	Ratio of $^R/_L$
	Per min.	Contractions	Contractions	
22° C.	19.6	5 =	11.7	2.34
a	30.0	8 =	19.5	2.44
b	40.0	10 =	20.6	2.06
c	52.0	10 =	21.8	2.18
d	60.0	10 =	24.5	2.45
e	34.0	10 =	19.6	1.96
f	24.0	5 =	12.3	2.46
g	20.0	3 =	6.8	2.26
h	5.7	2 =	4.4	2.2

If the ascidian heart be divided by median section into two equal parts, the pieces will both beat, the waves running from the ends toward the plane of section, and the rates will be approximately equal in the two halves, although the pieces will not necessarily be beating in the same phase. When our "ascidian" preparation of the turtle's heart is divided in this manner, we have the condition of right and left halves considered above — the two halves beat at *different* rates. The "ascidian" preparation may be manipulated in other ways to demonstrate the difference existing in the rhythmicity of the right and left sides; for example, one can clamp off or cut away the veins and sinus of the right leg of the preparation, whereupon, after a temporary stand-still, the preparation again begins to beat, but the direction of progression of the wave has been reversed; it now passes from left to right, and the rate is slower than normal — it is the rate of the left side.

Far more striking and beautiful is the experiment in which the right vagus nerve of an "ascidian" preparation is stimulated. In such an experiment the whole basal part of the right side, including veins, sinus, and auricular portion of the preparation, are inhibited; as in the previously described case, the veins of the left side now initiate a slower rate and the wave progresses from left to right involving the ventricles, but is lost in the auricle of the right half of the preparation, *i. e.*, when the wave meets tissue under vagus influence.

An attempt was made by the author to reverse the direction of beat in the "ascidian" preparation by altering the temperature of the venous ends of the preparation by the use of galvanic resistance wire after the method of Engelmann.[9] In the frog it is a simple matter to thus start a beat at a localized heated area on any of the basal veins, but the method is far less certain in the turtle heart owing to the difference in the rhythmicity of the veins of the two sides; if, for example, the ratio is $^{R}/_{L} = 4.1$, it follows, from the large amount of work which has been done on the question of temperature coefficient, and heart rate, that a difference in temperature between the right and left sides of our preparation of approximately 20° C. must be maintained.[10] This was difficult to accomplish in our laboratories with the heart *in situ*, but was readily effected in hearts removed from the body if they had been successfully split, *i. e.*, without the presence of "blocks" due to the section. The simplest manner of proceeding in these cases was to suspend the preparation by the ventricle and allow the right and left halves to depend into beakers of Ringer's solution of the proper temperatures. Thus, if the room temperature be 22° C. at both ends, the wave will pass from right to left, but if the temperature of the left limb be made 30° C. and that of the right 10° C., the wave will pass from left to right. Finally, under this head it should be stated that in one instance I attached a writing lever to the vein of the left side of the "ascidian" preparation, the mechanical stretching thus effected sufficed to raise the excitability to a point at which the impulses generated on the left side had a more rapid rate than the rate of discharge of the veins of the right side, a consequent reversal of the direction of the wave took place. The direction of the wave could be controlled at will by the application of or removal of the stretching due to this lever.

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[9] Engelmann: Archiv für die gesammte Physiologie, 1896, lxv, pp. 131–132.

[10] C. D. Snyder: University of California publications, Physiology, 1905, ii, p. 125, and Archiv für Physiologie, 1907, p. 118; T. B. Robertson, Biological bulletin, 1906, x, p. 242.

Having established, by these methods, the fact that *there exists a marked difference in the inherent rhythmicity of the two sides of the base of the heart*, and having proven that the beat normally originates in the veins of the right side near their ostia, we are in a position to give the results and interpretation of our investigations on the different behavior of the right and left vagi of the turtle — results which give us the solution of the problem which we have set before us.

THE ACTION OF THE VAGI.

In every individual examined in the course of this investigation it was possible to demonstrate a quantitative difference in the action of the two vagi. For a true conception of the variations in the action of these two nerves it is necessary to examine the effects of each upon the different parts of the heart, auricles, sinus, and veins, and also to examine the effects upon the lateral halves of the heart. It is further necessary to make this examination not only in the intact heart but also upon the different parts beating after physiological isolation from the rest of the heart. Gaskell [11] found that the right vagus only had a chronotropic effect, but that both vagi affected the auricle. Mills discovered what had escaped Gaskell, *viz.*, a unilateral action of the vagi exerted upon both the auricles and the basal veins, but Mills made no graphic record of his experiments, and thus overlooked many important points, in fact the very points which give the true explanation of the differences existing between the action of the two nerves.

In many instances *both* vagi will *apparently* produce the same inhibitory effect upon the heart, but if tracings are taken of the right pre- and post-caval veins and of the right auricle it is easily seen that the cause of the cardiac quiescence is usually quite different for the two vagi. The right vagus exerts a chronotropic effect, as well as other effects, upon the right veins and produces quiescence of the auricles and ventricles mainly through its chronotropic effects, rarely, with weak stimuli, the effect is brought about by production of a sino-auricular block, although in these cases the inotropic and other effects are also apparent. When the *left* vagus causes quiescence of the auricles and ventricles, it does so in a totally different way. The veins of the right side are usually unaffected; they continue to beat with their normal rate and force, but the left vagus decreases the excitability, conductivity, and contractility of the auricles to a point at which they fail to respond to the stimulus of the pace-maker, thus no impulses can pass through to the ventricle. Fig. 6 illustrates this point, the tracings being taken from the right veins and right auricle; the latter is seen to come to a complete stand-still while the former are entirely unaffected; the ventricles also ceased beating. Had a tracing of the left vein been taken it would have been found to have been affected precisely as were the auricles. A homolateral effect upon the basal veins was thus demonstrated. Similarly the difference in the action of the two vagi upon the right caval veins is well illustrated in Fig. 7, taken from the right veins of the intact heart. The left vagus was stimulated with strong interrupted shocks (4 cm.); only a loss of tone is evident, which was due to the effect upon the left vein (not registering), but there was no effect upon the rate or strength

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[11] GASKELL: *Loc cit.*, Fig. 5, Plate III.

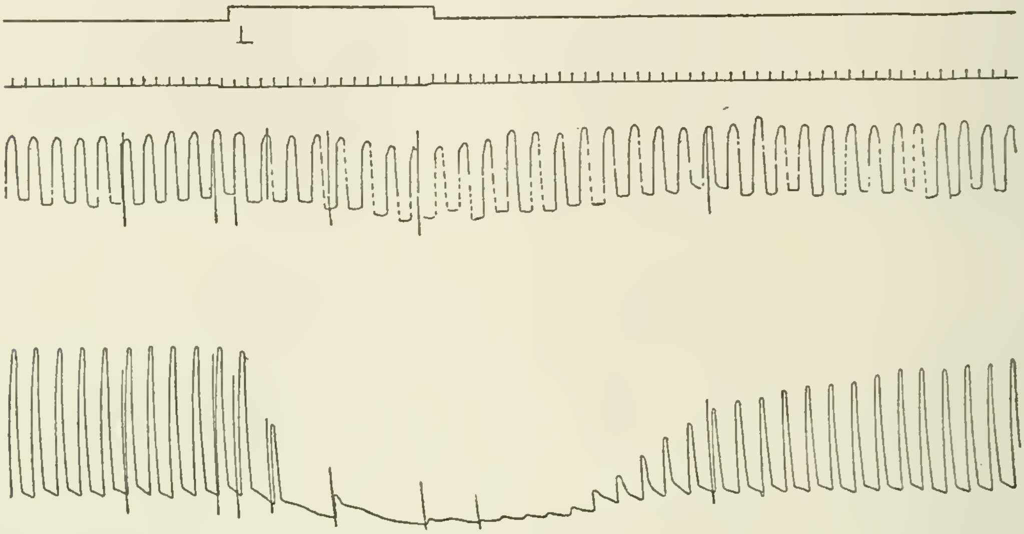


FIGURE 6. — Four fifths the original size. Simultaneous tracing of the right auricle (lower curve) and the right post caval vein (upper curve). At *L* the left vagus was stimulated, no effect was produced upon the right veins, but the effects on the auricles are marked and the ventricle ceased beating. For further explanation see text. Time in seconds.

of the right veins. When the right vagus was stimulated with *weak* interrupted shocks (11 cm.), the right veins were at once inhibited.

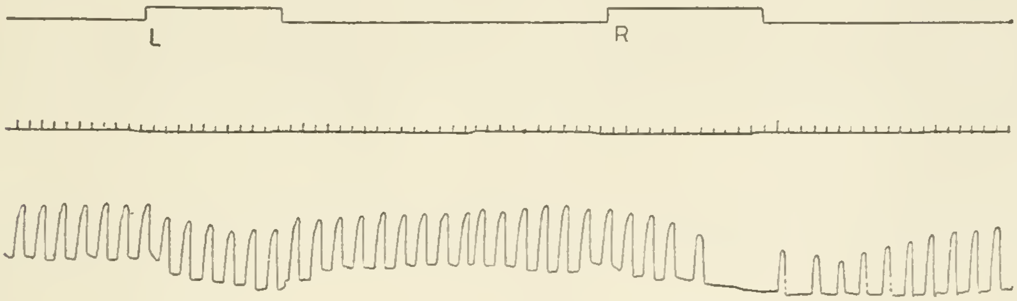


FIGURE 7. — Four fifths the original size. Tracing of the right caval veins showing that the left vagus (stimulated at *L*) is ineffective, while the right vagus, stimulated at *R*, is effective upon this cardiac "pace-maker."

The homolateral effects of the vagi on the veins is demonstrated by

CORRECTION. — The legend of Fig. 8 should read:

FIGURE 8. — One half the original size. Tracings taken from the right caval veins (lower curve in *A*, *B*, *C*, *D*) and left caval vein (upper curve) to show the homolateral effects of stimulation of the vagus. *R* and *R'* = stimulation of the right vagus with weak and strong currents respectively. At *L* and *L'* the left vagus was stimulated by weak and strong currents respectively. Further explanation in the text.

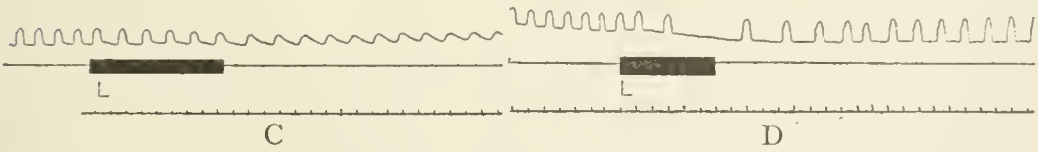
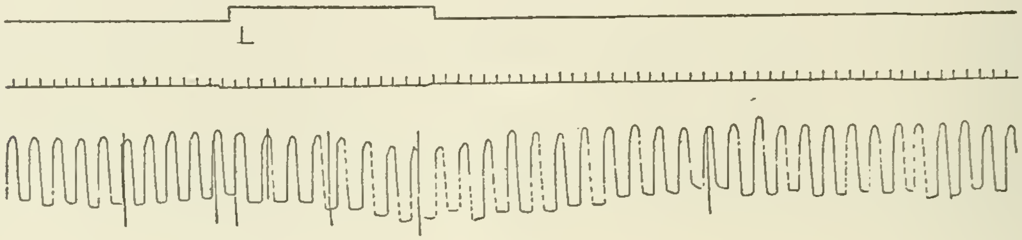


FIGURE 8. — One half the original size. Tracings taken from the right caval veins (upper curve in *A*, *B*, *C*, *D*) and left caval vein (lower curve) to show the homolateral effects of stimulation of the vagus. *R* and *R'* = stimulation of the right vagus with weak and strong currents respectively. At *L* and *L'* the left vagus was stimulated by weak and strong currents respectively. Further explanation in the text.

as a result of compression between them. The right vagus stops the beat of the right vein, as expected. It is readily seen, however, that the left vagus also exerts all of the typical vagus effects on the and ventricles mainly through its chronotropic effects, rarely, with weak stimuli, the effect is brought about by production of a sino-auricular block, although in these cases the inotropic and other effects are also apparent. When the *left* vagus causes quiescence of the auricles and ventricles, it does so in a totally different way. The veins of the right side are usually unaffected; they continue to beat with their normal rate and force, but the left vagus decreases the excitability, conductivity, and contractility of the auricles to a point at which they fail to respond to the stimulus of the pace-maker, thus no impulses can pass through to the ventricle. Fig. 6 illustrates this point, the tracings being taken from the right veins and right auricle; the latter is seen to come to a complete stand-still while the former are entirely unaffected; the ventricles also ceased beating. Had a tracing of the left vein been taken it would have been found to have been affected precisely as were the auricles. A homolateral effect upon the basal veins was thus demonstrated. Similarly the difference in the action of the two vagi upon the right caval veins is well illustrated in Fig. 7, taken from the right veins of the intact heart. The left vagus was stimulated with strong interrupted shocks (4 cm.); only a loss of tone is evident, which was due to the effect upon the left vein (not registering), but there was no effect upon the rate or strength



of the right veins. When the right vagus was stimulated with *weak* interrupted shocks (11 cm.), the right veins were at once inhibited.

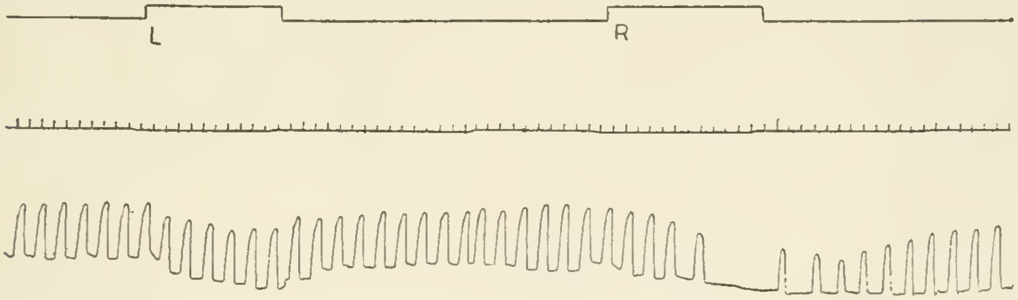


FIGURE 7. — Four fifths the original size. Tracing of the right caval veins showing that the left vagus (stimulated at *L*) is ineffective, while the right vagus, stimulated at *R*, is effective upon this cardiac "pace-maker."

The homolateral effects of the vagi on the veins is demonstrated by these tracings and is especially well seen upon referring to Figs. 4 and 8. As shown above, the veins in Fig. 4 are beating independently

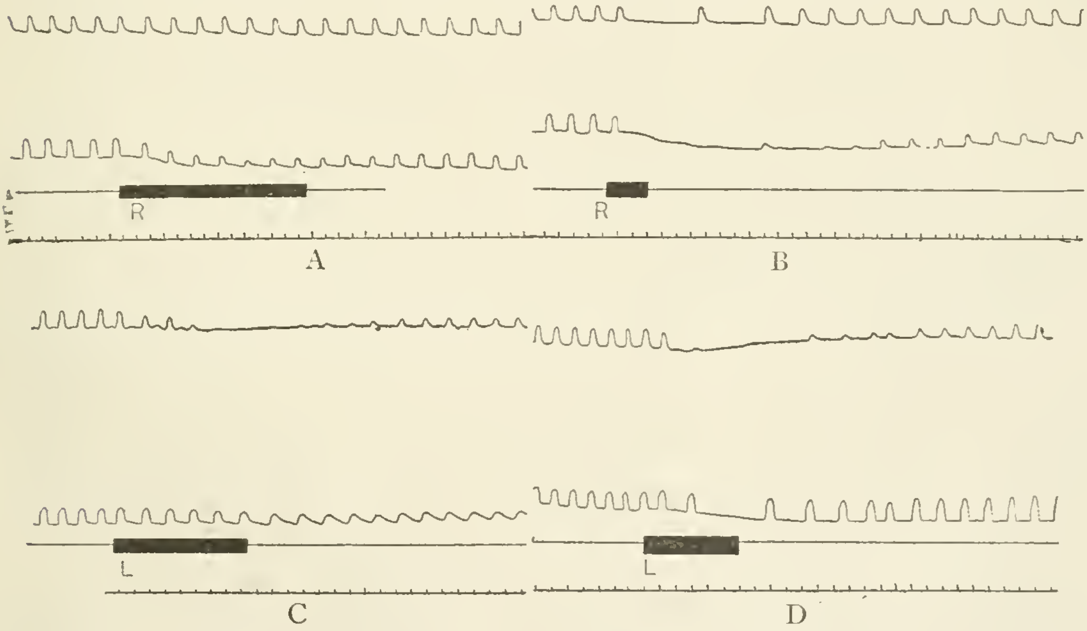


FIGURE 8. — One half the original size. Tracings taken from the right caval veins (upper curve in *A*, *B*, *C*, *D*) and left caval vein (lower curve) to show the homolateral effects of stimulation of the vagus. *R* and *R′* = stimulation of the right vagus with weak and strong currents respectively. At *L* and *L′* the left vagus was stimulated by weak and strong currents respectively. Further explanation in the text.

as a result of compression between them. The right vagus stops the beat of the right vein, as expected. It is readily seen, however, that the left vagus also exerts all of the typical vagus effects on the left vein, although a test upon the intact heart failed to reveal any marked chronotropic changes resulting from stimulation of this nerve.

It must not be inferred from what has been said in this discussion that the unilateral effects are absolute and invariable, although Mills stated this to be the case; while it is true that in many instances we are unable to demonstrate any crossed effects upon the basal veins, in many other cases they can be demonstrated, but in these latter instances a much stronger stimulation of the vagus nerve is required. These points are illustrated in Fig. 8, *A*, *B*, *C*, and *D*, the tracing being taken from the right and left veins of the intact heart. In *A* and *B* the action of the right vagus is shown when stimulated with weak (*R*) and strong (*R′*) induced shocks respectively. It is noted in both these tracings that the preponderant effect was on the homolateral vein, as is indicated by the effect upon the height of contraction. In *C* and *D* of the same figure the effects of stimulation of the left vagus are seen; with a weak current (*L*) the effect was confined almost exclusively to the homolateral vein, while the stronger stimulation sufficed to produce crossed effects to the right side as well and to induce thereby chronotropic effects. These four tracings from the veins illustrate indubitably the fact that the preponderant action of the vagus is homolateral, but they show also that crossed effects are possible, at least when the vagus stimulation is very strong, and show also that in addition to a cardiac quiescence produced by an effect solely upon the auricles (*cf.* description of Fig. 6, p. 342) the left vagus, in some instances at least, may produce true chronotropic effects if the stimulus is sufficiently strong. It may be well to state that these crossed vagus effects are the result of a crossing which takes place wholly within the substance of the heart itself; there is no crossing of impulses from side to side through an extra-cardiac plexus as in mammals, for it is possible to make a median sagittal section through all body tissues except the heart and still not interfere with such crossed effects as are possible.

The limits of this paper do not permit of extensive speculation on the question of how the crossed effects are produced, but it is certain that muscular fibres are affected by strong stimulation which are not involved by weaker stimulation of the vagi. And it would certainly be most interesting to learn whether the stronger stimulation involves more fibres in the vagus trunk, or whether new paths in the post-ganglionic plexus are broken into, as in irradiation in the central nervous system. These experiments open up another important question whether the weakening of the contraction resulting from weak vagus stimulation is ever due to a slight involvement of all the muscle fibres, or whether only a part of the musculature is involved in the inhibition while the rest remains unaffected and beats with full force. It certainly looks as if the latter were the case. Again, if the effect of varying the strength of nerve stimulation depends upon a variation in the number of vagus nerve fibres involved, future investigation may well show that the "all or none law," as applied to the heart, may also apply to the contraction of individual fibres of skeletal muscle.

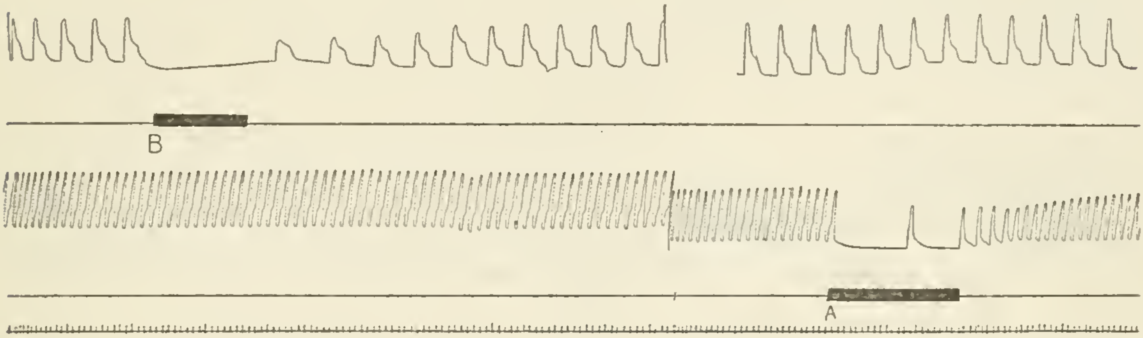


FIGURE 9. — One half the original size. Tracing of the right (lower curve) and left auricles (upper curve) after splitting sagittally into lateral halves. At *A* the right vagus was stimulated, at *B* the left vagus. The latter showed typical chronotropic and other effects upon the spontaneous beats of its homolateral half; previous to splitting this nerve produced no chronotropic effects upon the heart beat.

It was demonstrated above that even in those cases in which strongest stimulation of the left vagus failed to stop the heart, typical chronotropic effects of this nerve could be induced on the left vein if it were cut away from the heart and thus beat in its own rhythm; the same fact is beautifully demonstrated by the use of the "split" heart prepared as described above (page 336). Fig. 9 represents a tracing from such a preparation, the levers being attached to the auricles. In this experiment it was not possible to stop the intact heart by stimulation of the left vagus. It is seen, however, that when the left half initiated its own rhythm the left vagus had the same effect upon it that the right vagus had upon the right half. It is clear from these experiments that *both vagi are active when the portions of the heart which they individually innervate initiate their own rhythm.*

The conception of a more or less sharply defined homolateral and local action of the vagi is more strongly emphasized if we study the action of each vagus upon its corresponding auricle. Here, again, the reference to tracings makes conditions most clear. In Fig. 10 tracings are taken with the levers of like dimensions attached to the auricles. The left vagus was stimulated at *A* with the secondary coil at 4 cm.; even this strong current produced no change in the rate, but only tonus changes in the height of contraction, the effect upon the left auricle being much more pronounced than upon the right. When the right vagus was stimulated at *B* with a much weaker current (secondary at 9 cm.), a change in rate was effected as a result of action on the veins of the right side; at the same time the decrease in height of contraction was much more pronounced upon the right auricle than upon the left. Similar effects were noted in all other tracings made by the author; the effects were found to be more pronounced and to last longer on the auricle homolateral to the vagus stimulated, even when, for comparison, the strength of stimuli applied to the two nerves was equal. Another experiment was devised to show the effects of stimulation of the vagi with single induction shocks thrown in at different intervals. The graphic results are not shown, but an analysis of the tracings is given in Table II.

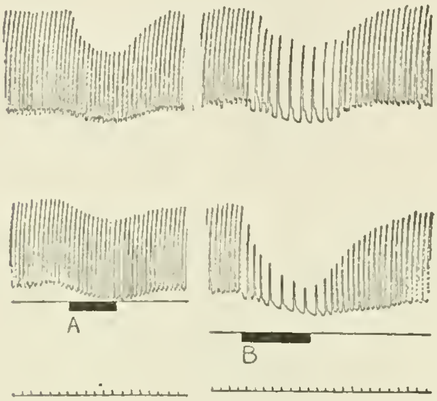


FIGURE 10. — One half the original size. Tracings of the auricular contractions showing the homolateral effects of the vagi. Upper, from the left auricle; lower, from the right auricle. *A* = stimulation of the left vagus; *B* = stimulation of the right vagus.

It follows from this analysis that the homolateral effect of each vagus is decidedly greater than the contralateral. This is best shown under the effects of left vagus stimulation because the rate and regularity of contraction are unaffected. Although the greater effect is shown on the side of the vagus stimulated, the experiments also bring out clearly that in the auricles as in the veins and sinus the vagus effects may cross to the contralateral side, which is contrary to the statement made by Mills (*loc. cit.*). In the experiment just analyzed the strongest induced shocks were utilized.

The above considerations put us in a position to amplify the interpretation of the action of the left vagus made above. In all of those cases in which the left vagus seems to have no effect upon the heart, examination shows that in reality it does decrease partially or completely the contraction of the homolateral part of the base of the heart (left vein and left part of the sinus); it does also affect profoundly the height of contraction of the left auricle, and to a lesser extent that of the right auricle; the rate of contraction is not affected in these cases because the left vagus does not innervate the pace-maker (right veins), and the auricles, or at least the right auricle, is still able to conduct impulses to the ventricle in spite of the evident effects of the vagus. If we examine critically those cases in which the left vagus stops the heart, we find that it does so in a very different way from that in which the right vagus acts, for we find, as noted above, that the right veins in reality continue to beat in the

TABLE II.

RIGHT VAGUS STIMULATED.

Stimulation interval.	Right auricle (height decreased).	Per cent decrease.	Left auricle (height decreased).	Per cent decrease.
1	Complete quiescence	..	Complete quiescence	..
2	From 18 mm. to 10 mm.	44	From 19 mm. to 15 mm.	21
5	From 24 mm. to 18 mm.	25	From 20 mm. to 18 mm.	10
10	From 21 mm. to 18 mm.	14	From 20 mm. to 18 mm.	10
20	Well-marked waves	..	Waves very slight	..
	LEFT VAGUS STIMULATED.			
1	From 20 mm. to 10 mm.	50	From 20 mm. to 3 mm.	85
2	From 21 mm. to 14 mm.	33	From 20 mm. to 8 mm.	60
5	From 23 mm. to 18 mm.	21	From 21 mm. to $10\frac{1}{2}$ mm.	50
10	From 23 mm. to 20 mm.	13	From 21 mm. to 14 mm.	33
20	Most marked waves	..	Waves less marked	..

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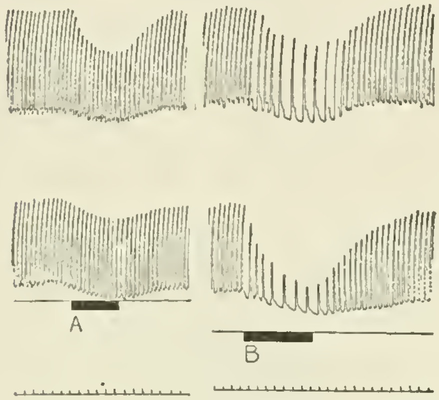

FIGURE 10. — One half the original size. Tracings of the auricular contractions showing the homolateral effects of the vagi. Upper, from the left auricle; lower, from the right auricle. *A* = stimulation of the left vagus; *B* = stimulation of the right vagus.

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5	From 23 mm. to 18 mm.	21	From 21 mm. to 10½ mm.	50
10	From 23 mm. to 20 mm.	13	From 21 mm. to 14 mm.	33
20	Most marked waves	..	Waves less marked	..

localized and terminal, and are exerted upon the contracting muscle wholly independent of any effects upon the region of the heart which is responsible for the inception of rhythm and rate — a condition which is very different from that which has been shown by Carlson to exist in Limulus.[19]

SUMMARY.

1. The rhythmicity of the veins entering the right side of the basal portion of the turtle's heart is greater than that of the vein of the left side. When isolated from the heart, the right veins may beat from two to four times as fast as the left. Similarly, when the heart is cut sagittally into lateral halves, the right half beats faster than the left.

2. The beat of the turtle's heart normally starts in the veins of the right side; a distinct pause marks the progress of the contraction wave at the right veno-sinus junction, and another pause intervenes as the wave passes from sinus to left vein. Thus the veins must be looked upon as separate tubular heart cavities, physiologically as distinct as is the sinus.

3. When the heart is split sagittally, the rhythm of the left half is initiated in the vein of that side, and is slower than that of the right half.

4. The vagi of the turtle, when stimulated, show a preponderant homolateral effect which is most pronounced upon the basal veins — less so in the auricles. Crossed effects may be obtained in some cases, even upon the basal veins, when the vagi are strongly stimulated.

5. The left vagus is less effective upon the normal rhythm of the heart than is the right vagus. This is due to the fact that it does not innervate the right vein which initiates the cardiac rhythm.

6. In those cases in which the left vagus fails to show chronotropic effects upon the intact heart it can still be shown to produce quiescence of the left vein and left auricle. Furthermore in these same

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[19] These localized effects are chronotropic if they affect the fastest beating portion of the heart — and then only — but may affect the rate of any part of the heart which beats automatically and is innervated by the vagus. The details of the chronotropic effects of the vagi on different parts of the heart are now being investigated further.

cases, if the heart be split sagittally and the left half beats, the left vagus is found to produce typical *chronotropic* as well as other effects.

ADDENDUM. — Since the above article went to press the author has had the opportunity, through the courtesies extended him at the U. S. Fisheries Laboratory at Beaufort, N. C., of conducting investigations upon three species of marine turtles, *viz.*, Chelonia mydas, Colpochelys kempi, and Caretta caretta. All three of these species show the vagus fibres of the right side to be the more effective. The enormous size of these hearts makes it easy to determine the point of origin of beat to be in the right veins. The "split" heart and "ascidian" heart preparation can be made without functional derangement or production of blocks. All experiments confirm the results detailed in the body of this article.

REGENERATION OF AUERBACH'S PLEXUS IN THE SMALL INTESTINE.

By WALTER J. MEEK.

[*From the Physiological Laboratory of the University of Wisconsin.*]

IN a previous communication[1] on this subject from Dr. Carlson's laboratory, it was shown that after transection of the small intestine, physiological restoration, as determined by the passage of a peristaltic wave across the lesion, could be demonstrated as early as the eighth day. On closer analysis this peristaltic wave was found to depend for its transmission on a purely mechanical factor, the tug of the musculature at the line of transection. The passage of a wave of contraction was thus shown to be insufficient as a test for the regeneration of the nervous motor mechanism of the intestine. Recourse was then had to histological methods. By means of the gold chloride stain regeneration of certain fibres in Auerbach's plexus was shown in one of six dogs, one hundred and eighty days after transection. Unfortunately the origin of the fibres was not definitely known. Instead of being outgrowths of nerve cells in the intestinal plexus, they might have been postganglionic axones from extrinsic abdominal ganglia.

The problem has been taken up anew in hopes of being able to demonstrate conclusively a regeneration of the plexus of Auerbach accompanied by a return of complete physiological function. Regeneration has therefore been tested both histologically and physiologically. Histologically the gold chloride stain was again used and reduction brought about in arsenic acid. A more complete description of the technique will be found in the protocols.

Physiologically regeneration has been tested by the passage of a wave of inhibition through the transected areas. Bayliss and Starling[2] in their well-known papers have shown that the contraction wave in the intestine is a complicated phenomenon, the musculature being contracted above and inhibited for some distance below the point of stimulation. All investigators agree in assigning this complex reflex to the intrinsic nervous mechanism of the intestine. The reflex remains when the mesenteric nerves are degenerated.[3] Pithing the cord does not eliminate it. It is inconceivable, as Bayliss and Starling have remarked, that muscular tissue could respond on one side of the stimulation by contraction and on the other by inhibition. Our own experiments show that the return of conduction for this wave of inhibition takes place at a time much later than the regeneration of the muscular coats. And finally Magnus[4] found that the law of the intestine could not be demonstrated if the plexus of Auerbach were stripped off with the longitudinal muscular coat. It would seem therefore beyond question that the conduction of this wave of inhibition is taken care of by Auerbach's plexus, and the occurrence of this wave would therefore demonstrate the continuity of that nervous mechanism.

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[1] MEEK: This journal, 1910, xxv, p. 367.

[2] BAYLISS and STARLING: Journal of physiology, 1899, xxiv, p. 99.

Methods.

Cats and dogs were again used in the experiments. These animals had the small intestine transected and were then allowed to live from nineteen to two hundred and forty days, when they were used to test the passage of a wave of inhibition across the lesion. In our previous work the muscular coats had been cut down to the mucosa and an end to end anastomosis made. In this way the lumen of the intestine was unopened, an apt union of the layers was insured, and only a small amount of connective tissue developed in the scar. This method was again used on the cats.

In the experiments on dogs a different technique was employed. Dr. S. A. Matthews had noted in some intestinal work that a glass tube tied into a duct or loop of intestine in time passed out, the ligatures that held it having gradually cut through the tissues. This suggested that transection might be brought about by tying glass tubes in the intestine. A piece of glass tubing 5 cm. long and 1 cm. in diameter with the ends double flanged was slipped into the lumen of the intestine and then securely tied in place by means of two ligatures which encircled the gut exactly over the flanges. Silk twist, size C, was found convenient for the ligatures. Being tied tightly, the ligatures gradually transected the gut, and when they finally cut through the walls of the intestine the glass tube was of course freed and passed out with the feces. The tube was usually recovered at the end of seven days. The intestinal contents pass through a tube in such a position readily, and no obstruction was ever apparent. To make sure of an abundance of material two of the dogs were subjected to the procedure twice, making four transections in each.

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[3] Langley and Magnus: Journal of physiology, 1905, xxxiii, p. 34.

[4] Magnus: Archiv für die gesammte Physiologie, 1904, cii, p. 123.

This method of section is especially suitable for regeneration work. The layers are left exactly end to end and a minimum of scar tissue is produced. So little scar tissue is found and so scant are the adhesions that in one dog which had lived two hundred and forty days after the operation, the lines of transection could not be located until the intestine was opened and the mucosa scraped away. The line of section then showed as a white line around the intestine. In all other cases this white ring marking the lesion was apparent in the living animal, and by means of it and the recognition stitches absolute identification of the place was possible.

Records of the intestinal movements were made with a balloon and a Marey tambour. The balloon consisted of thin rubber fastened over the end of a small curved glass tube, all of which could be inserted into a coil of the gut so that the balloon rested a few centimetres below the line of section. The balloon was connected by rubber tubing to a recording tambour and to a small atomizer bulb which was used to raise the pressure. A manometer was not kept in connection, but the pressure was about 10 to 15 cm. of water. When the balloon was placed in the proper position, the lever of the tambour recorded the rhythmical contractions of the circular muscular coat. By stimulating a point above the balloon the rhythmical movements were at once abolished, which gave graphic evidence that a wave of inhibition had swept down the intestine.

Description of Experiments.

The first series consisted of five cats. These animals were used to find out whether complete physiological regeneration ever occurred, and if so at approximately what time after the transection. Cats

Nos. 1, 2, and 3 were operated on as described above, and kept for one hundred and eighty, two hundred and eleven, and two hundred and forty days respectively. One of the protocols will best illustrate the entire procedure.

Cat No. 2. — August 15. Intestine transected by cutting the muscular coats down to the mucosa. Rapid recovery.

March 18. Cat given 20 c.c. castor oil.

March 19. Two hundred and sixteen days later tested for passage of wave of inhibition across the line of transection.

9.15 A. M. Ether anæsthesia. Tracheotomy.

9.30 A. M. Cat pithed. Cord entered in upper thoracic region.

9.35 A. M. Skin of abdomen opened along median line, reflected, and sewed to an iron ring. Cavity thus formed filled with warm saline. Abdomen opened and loop with transection drawn out.

9.50 A. M. Balloon placed in gut well below line of section. Circular muscles immediately began to show typical rhythmical movements.

9.55 A. M. Pinched intestine with forceps 5 cm. above lesion. Slight relaxation in parts surrounding the balloon.

10.11 A. M. Crystal of salt placed on intestine 2 cm. above section. No result except a marked local contraction.

10.19 A. M. Pinched lightly 2 cm. above section. Muscle around balloon inhibited ten to fifteen seconds, when rhythmical movements again gradually returned. (See Fig. 1.)

10.37 A. M. Salt crystal again applied above lesion. Marked constriction with inhibition over balloon.

10.42 A. M. Pinched above line of transection. Contraction at point stimulated and inhibition shown by balloon.

10.54 A. M. Cat used for injections not bearing on this problem.

From the protocol it will be seen that rhythmical contractions of the circular muscular coat below the line of transection were abolished by stimulation above. Fig. 1 illustrates this point. Cats Nos. 1 and 3 gave similar results. In each one a wave of inhibition was shown to pass the lesion. It is not thought necessary to give the protocols, since they are so nearly identical with the preceding. Intestinal movements are demonstrated with some difficulty in cats, but these rhythmical movements of the circular coat with inhibition below the point of stimulation were shown in every animal with ease. Giving castor oil to clear the intestines and eliminating extrinsic nervous effects by pithing as suggested by Bayliss and Starling make the results almost certain. It will be noted that results were not so consistent when salt was used as a stimulus. In later experiments this method of stimulation was discarded, and pinching lightly with a blunt pair of forceps was used exclusively.

It would seem clear from the experiments just cited that a complete physiological regeneration occurs, and it may be looked for at least as early as the one hundred and eightieth day. The next point undertaken was to show that this complete return of function is associated with a tissue regenerating much more slowly than muscle, that is, the nerve plexus. Previous work [5] has shown that the longitudinal coat of the cat's intestine may be almost perfectly renewed in nine days. Sections made from seventeen days on present a picture of complete muscular regeneration. Two cats, Nos. 4 and 5, were accordingly tested for passage of the inhibitory wave nineteen and forty days after transection. In each of these animals rhythmical contractions below the line of transection could not be inhibited by stimulation above. The protocols are unnecessary, since the technique was precisely the same as in the previous experiments. Fig. 2 is a tracing from Cat No. 5. The balloon was 5 cm. below the transection. At the signal marked 4 the gut was pinched 2 cm. above the lesion. No inhibition occurred. At the signal marked 5 a stimulus was applied as closely as possible below the line of section. As can be seen, a marked inhibition of the rhythmical contractions at once resulted. In both animals these results were obtained repeatedly, and not a single time did the wave of inhibition break through. We

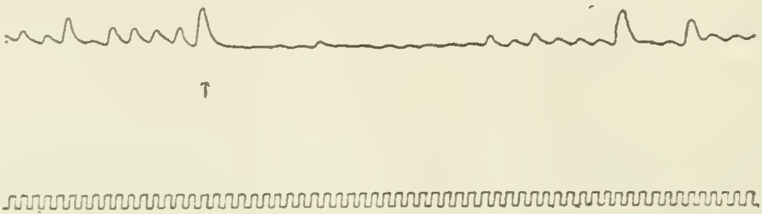

FIGURE 1. — Showing the passage of a wave of inhibition across the line of transection in a cat two hundred and sixteen days after the transection was made. The arrow shows stimulation above the line of section.

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[5] MEEK: *Loc. cit.*

have seldom succeeded in staining the plexus of the cat intestine very successfully, and for that reason an histological examination was not made in the preceding experiments.

It would be interesting but relatively unimportant to know just what interval is required after transection to make regeneration of function complete, but no attempt was made to answer that question. The work seems to show conclusively that complete return of function is associated with some tissue regenerating after the fortieth day, presumably the nerve plexus.

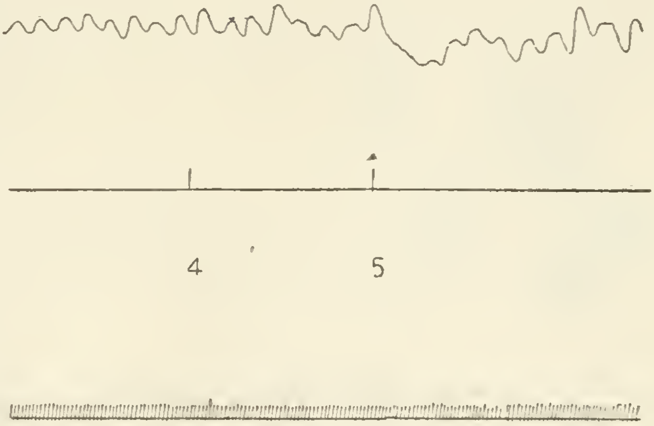

FIGURE 2. — Showing the failure of inhibition to pass the line of transection in a cat forty days after the transection was made. At 4 the intestine was stimulated above the transection. The rhythmical movements continue. At 5 a stimulus is applied just below the transection. The rhythmical contractions are at once abolished.

The second series of experiments was carried out on four dogs. These animals had the intestines transected by means of ligatures tied around glass tubes as previously described, and they were then allowed to live two hundred and thirty, two hundred and twenty-five, one hundred and sixty-seven, and one hundred and twenty-two days respectively. The purpose of these experiments was to show the passage of the inhibitory wave across the line of transection in the dog, and in the same specimen demonstrate histologically the regeneration of plexus fibres. The plexus of the dog fortunately is usually stained

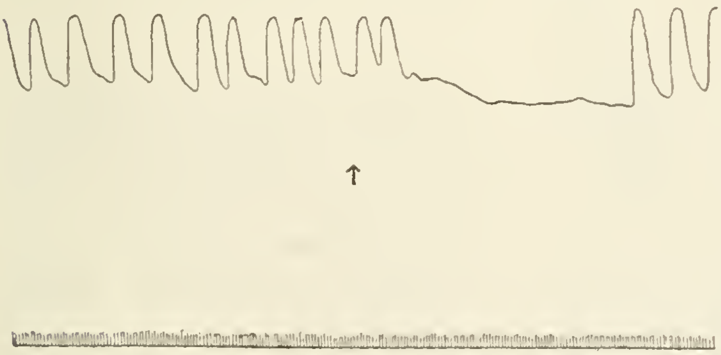

FIGURE 3. — Showing passage of the wave of inhibition across the line of transection in a dog two hundred and thirty days after the transection was made. At the signal the intestine was stimulated by pinching 4 cm. above the section.

with little difficulty. The protocol for Dog No. 1 will illustrate the entire procedure and present the results at the same time.

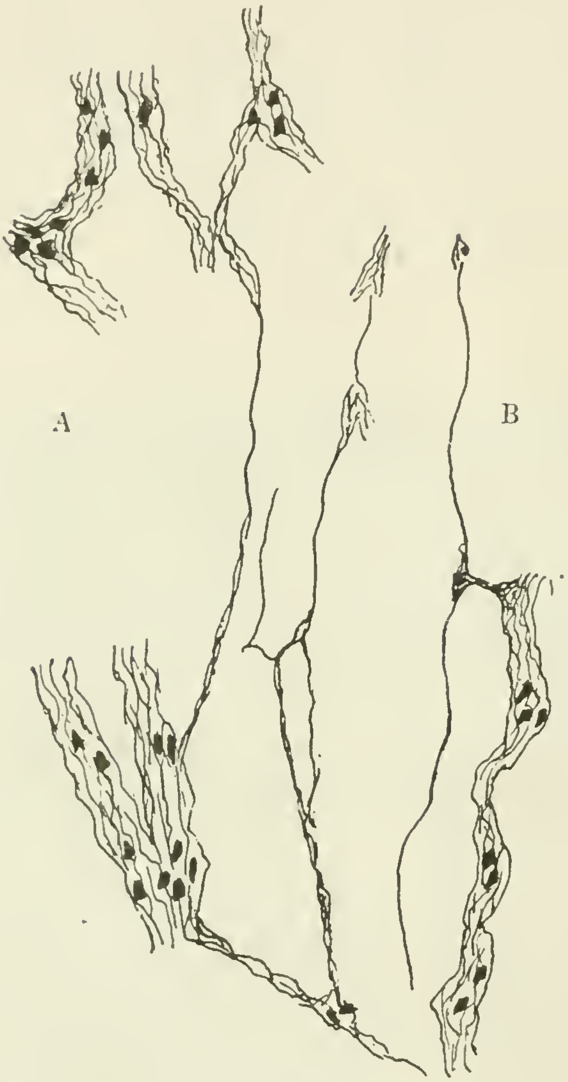

FIGURE 4. — Gold chloride stain showing regeneration of nerve fibres in plexus of dog two hundred and thirty days after transection. *A* to *B* marks the line of section.

Dog No. 1. — August 19. Intestine transected by inserting a glass tube and ligating around the intestine.

April 6. Dog given 30 c.c. castor oil.

April 7. 1.45 P. M. Given 1 grain morphine.

2.30 P. M. Ether anæsthesia. Tracheotomy. Skin of abdomen opened, reflected and sewed to an iron ring. Cavity thus formed filled with warm saline. Abdomen opened and loop of intestine with transection drawn out.

2.50 P. M. Balloon inserted in loop of intestine 6 cm. below transection. Rhythmical contractions of circular muscular coat at once recorded by tambour.

3.00 P. M. Stimulated by pinching 4 cm. above line of section. Contractions recorded by tambour at once inhibited.

3.05 P. M. Repeated above procedure with same results. (See Fig. 3.)

3.08 P. M. Repeated procedure with same results.

3.11 P. M. Repeated procedure with same results.

3.20 P. M. Cut longitudinal slit in intestine just above balloon to relieve air pressure.

3.22 P. M. Pinched 2 cm. above lesion. Contractions below transection at once inhibited.

3.30 P. M. Dog killed. Area with transection removed. Mucosa scraped off with scalpel. Placed in 0.5 per cent arsenic acid for thirty minutes, in gold chloride for forty-five minutes, and then reduced in 1 per cent arsenic acid over the water bath for fifteen minutes.

Fig. 3 shows the passage of a wave of inhibition across the transected area in the above experiment. In Fig. 4 is presented the appearance of a small portion of the transection after being treated with the gold chloride. Fibres may be seen crossing the scar tissue and entering the plexuses on either side. The findings just mentioned were exactly duplicated in two of the other dogs. No tracing was taken from Dog No. 2 because of some question as to the exact location of the transection, the recognition stitches for some reason having disappeared. The transected portions of all four dogs gave many examples of nerve fibres extending across the scars. The number of axones passing across amounts in itself almost to a demonstration of plexus regeneration. Fig. 5 is a tracing from Dog No. 4, one hundred and twenty-two days after transection. The balloon was well below the line of transection. At the signal marked 1 the intestine was pinched lightly 8 cm. above, and at the signal marked 2, 15 cm. above the transected area. The wave of inhibition

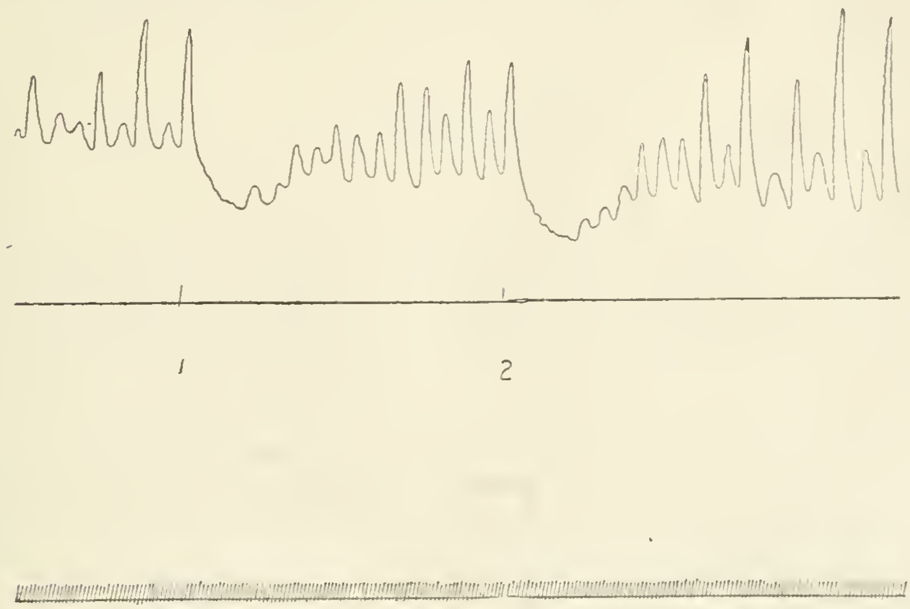

FIGURE 5. — Showing the passage of inhibition across lines of transection one hundred and twenty-two days after the transections were made. Stimulation at 1 was 8 cm. and at 2 15 cm. above line of section. In the case of 2 the wave of inhibition passes through two transections.

in this case passed down the intestine about 20 cm. and crossed two transections.

The above experiments seem to offer conclusive proof that in dogs as well as cats there is a complete return of physiological function after transection of the small intestine, and this return of function is accompanied by and no doubt depends on a regeneration of Auerbach's plexus.

SUMMARY.

The small intestine of cats and dogs was transected in order that regeneration of Auerbach's plexus might be tested at a later time.

Complete physiological regeneration as determined by the passage of a wave of inhibition across the line of transection has been demonstrated in three cats and three dogs. This return of function occurs in dogs as early as the one hundred and twenty-second day.

In four dogs this return of physiological function was shown to be accompanied by a growth of nerve fibres across the line of transection.

In cats it has been found that the wave of inhibition does not pass the line of transection until late in the course of recovery. Passage was impossible at the fortieth day, yet at this time muscular and epithelial regeneration is practically complete. Return of function is therefore associated with a slowly regenerating tissue, presumably the nerve plexus.

The above evidence we believe amounts to a demonstration of the regeneration of Auerbach's plexus in the cat and dog, accompanied by a return of complete physiological function.

THE CHEMICAL REGULATION OF VASCULAR TONE AS STUDIED UPON THE PERFUSED BLOOD VESSELS OF THE FROG.

By D. R. HOOKER.

[*From the Physiological Laboratory of the Johns Hopkins University.*]

THE substances investigated were perfused through the whole frog under a constant pressure. The inflow cannula was tied into the bulbus arteriosus, and the fluid as it escaped from cuts in the great veins collected at the corner of a glass plate, on which the frog was placed, and dripped onto a cover slip attached at the end of a tambour lever. A second tambour recorded the drops on a smoked drum. A Jacquet chronograph was used for a time record. The head of pressure in any one experiment was equalized for all solutions by adjusting the air-inlet tubes of the Mariotte flasks to a spirit level. The assumption was made that complete pithing of the spinal canal excluded the possibility of the effects being produced by action of the nervous system and that therefore the phenomena observed exhibited the reaction of the vascular musculature. It is proper to state, however, that such reaction may be due primarily to an effect upon the nerve endings rather than upon the muscle itself. An increase in the number of drops indicated a relaxation of vascular tone, and a decrease in the number of drops indicated an improvement of vascular tone.

Action of Sodium, Calcium, and Potassium Ions.

The influence and importance of calcium and potassium salts for the beat of the heart are now well established. Calcium acts to increase and potassium to decrease the cardiac tone. This antagonistic action of calcium and potassium has also been observed by Stiles for the musculature of the œsophagus[1] and stomach[2] of the frog, and by Mayer[3] and others for the neuro-muscular mechanism of marine invertebrates. It is not surprising, therefore, that the musculature of the blood vessels should exhibit a similar response.

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[1] Stiles: This journal, 1901, v, p. 338.

[2] Stiles: *Ibid.*, 1903, viii, p. 269.

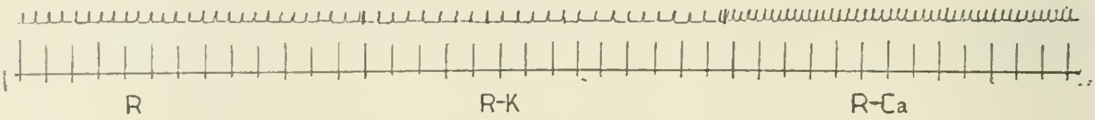

FIGURE 1. — Effect of removal of potassium and of calcium from Ringer's solution on vascular tone in the frog. Upper line, rate of perfusion in drops. The drum was stopped on changing solutions until conditions became constant. The lower line gives the time in seconds. R = Ringer's solution: R–K = Ringer's solution without potassium: R–Ca = Ringer's solution without calcium.

This response is most clearly seen by comparing the effect of a Ringer's mixture[4] with the mixture minus the calcium on the one hand and minus the potassium on the other hand (see Fig. 1). Thus in one experiment with 20 cm. water pressure these figures were obtained:

Solution.	Period.	Drops in 30 sec.	Period.	Drops in 30 sec.	Period.	Drops in 30 sec.	Period.	Drops in 30 sec.	Period.	Drops in 30 sec.
Ringer	1	15.7	4	16.5	7	21.0	10	18.9	13	21.6
Ringer minus calcium . .	2	17.4	5	15.0	8	31.5	11	33.0	. .	. . .
Ringer minus potassium .	3	11.1	6	10.5	9	13.4	12	18.9	. .	. . .

Relaxation of vascular tone is here indicated by an increase in the number of drops and *vice versa*. The removal of the calcium produced a decrease in tone five times out of six, and the removal of potassium caused an increase in tone six times without an exception. The accuracy of these results was confirmed on three other frogs.

It is possible also to demonstrate the specific effect of calcium and potassium by comparing a solution of sodium chloride with solutions

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[3] MAYER: Carnegie Institution Publication, 1910, No. 132, p. 1.

[4]

NaCl	0.07 per cent.
KCl	0.035 per cent.
$CaCl_2$	0.025 per cent.
$NaCO_3$	0.003 per cent.

of sodium chloride plus calcium chloride and of sodium chloride plus potassium chloride. To do this it is however necessary to considerably increase the amounts of calcium and potassium in solution (about six times the quantity used in Ringer's mixture). With such solutions the potassium causes a distinct decrease in tone compared with sodium as well as with sodium plus calcium. The effect of calcium is much easier of demonstration because it antagonizes both sodium and potassium (see Fig. 2). The predominating action of calcium is seen in a comparison of Ringer's mixture with sodium chloride. In a single experiment to confirm this point the sodium invariably relaxed the tone produced by the Ringer's mixture. In this case the calcium more than counteracted the effect of sodium and potassium together.

ACTION OF OTHER SUBSTANCES.

Oxygen and carbon dioxide. — Bayliss[5] has reported the observation that Ringer's solution saturated with carbon dioxide produces vascular relaxation when perfused through the limb vessels of frogs as compared with Ringer's solution not so saturated. It was not difficult to confirm this on the whole frog as used in these experiments. In order to investigate the specific action of oxygen, Ringer's solution was boiled in a flask to drive off as much of the oxygen as possible. The flask was then corked until the solution became cool. The solution was divided in three Mariotte flasks. Oxygen was bubbled through one of these and carbon dioxide through another for about ten minutes; the third bottle contained the solution qualitatively free from both oxygen and carbon dioxide. Obviously gas diffused into and out of the bottles freely so that the specific action of the gases could be demonstrated for only a short time. Under these conditions figures such as the following were obtained (see table on page 365, also Fig. 3):

It has been suggested that the carbon dioxide here acts as an acid.[6] Similarly it may be suggested that oxygen acts as an antacid in that it oxidizes the lactic acid formed by the tissues and so produces a

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[5] BAYLISS: Journal of physiology, 1901, xxvi, p. xxxii.

[6] BAYLISS: Ergebnisse der Physiologie, 1906, v,p. 319.

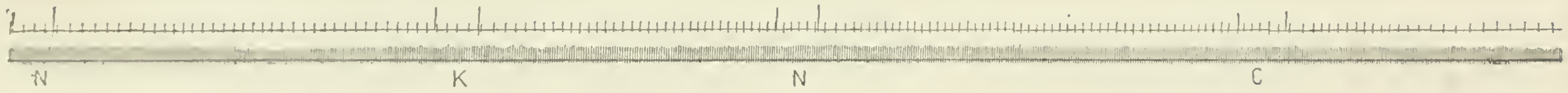

FIGURE 2. — About one half the original size. Specific effect of calcium and of potassium on vascular tone in the frog. Upper line, rate of perfusion in drops. The extended marks indicate the period of change from one solution to another. The lower line gives the time in seconds. N = NaCl 0.7 per cent; K = NaCl 0.7 per cent plus K Cl 0.21 per cent: C = NaCl 0.7 per cent plus $CaCl_2$ 0.17 per cent.

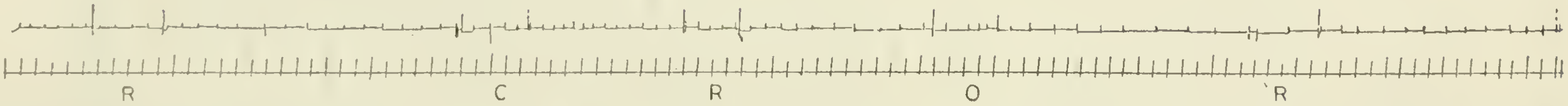

FIGURE 3. — About seven elevenths the original size. Effect of oxygen and carbon dioxide on vascular tone in the frog. Upper line, rate of perfusion in drops. The extended marks indicate the period of change from one solution to another. Lower line gives the time in seconds. *R* = Locke boiled; *C* = Boiled Locke saturated with CO_2; *D* = Boiled Locke saturated with O_2.

condition analogous to that brought about by the perfusion of alkaline solutions.[7]

The reaction of the smooth muscle of the blood vessels to oxygen and carbon dioxide here described is the exact opposite of that observed for the smooth muscle of the gastro-intestinal tract.[8] This difference in the response of anatomically similar tissue has been confirmed upon isolated bits of tissue (from both warm and cold blooded animals) from which graphic records were obtained and will be made the subject of a special communication. It may here be remarked, however, that the results obtained in the case of both the venous and arterial musculature appear to be directly opposed to the hypothetical action of carbon dioxide on venous tone as advanced by Henderson[9] in support of the acapnia theory of surgical shock. In the observations on isolated bits of tissue thus far made the circular ring of muscle was suspended in a moist chamber and submitted to pure atmospheres of oxygen and carbon dioxide. The *apparent* antagonism to Henderson's theory may be susceptible of explanation on the ground that the effect of saturated atmospheres on the tissue is different from what might result in a less abnormal gaseous environment. However this may be, the difference in the behavior of the vascular and intestinal musculature under exactly similar conditions points strongly to the fact that reasoning by analogy from the behavior of the intestines to the blood vessels is not justifiable. The present results are, furthermore, in agreement with the

Solution.	Period.	Drops in 30 sec.	Period.	Drops in 30 sec.	Period.	Drops in 30 sec.	Period.	Drops in 30 sec.
Ringer boiled	2	11	4	10	6	9.5	8	9
Ringer + CO_2	1	16	3	16	..	..	..	..
Ringer + O_2	..	..	..	..	5	7.0	7	7

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[7] Gaskell: Journal of physiology, 1880, iii, p. 48.

[8] Starling: Schäfer's Text-book of physiology, 1900, p. 331.

[9] Henderson: This journal, 1910, xxvii, p. 152; also Johns Hopkins Hospital Bulletin, 1910, xxi, p. 235.

findings of Jerusalem and Starling[10] that a certain amount of carbon dioxide (5-8 per cent of an atmosphere) increases the efficiency of cardiac muscle as exhibited in the isolated mammalian heart by increasing the diastolic relaxation as well as by increasing the systolic contraction.

Urea. — Urea is a waste product of the organism, and as such we should expect that it would be eliminated as quickly as possible. It is found in accordance with this expectation that urea when introduced into the circulation stimulates the heart to more efficient activity and at the same time causes a decrease of tone in the vascular musculature. Backman[11] in a series of experiments on the isolated rabbit's heart has shown that urea as well as other nitrogenous catabolic products (ammonium carbamate, ammonium carbonate, sodium hippurate, creatin, hypoxanthin, xanthin, etc.) have a stimulating action on the heart. There is an increase in both rate and force of beat, the latter apparently being due to increased diastolic relaxation as well as to increased systolic contraction as is the case with carbon dioxide in the observations of Jerusalem and Starling.

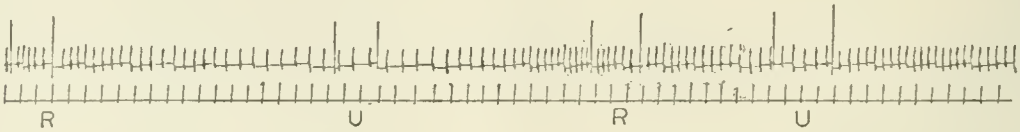

FIGURE 4. — Two thirds the original size. Effect of urea, 0.5 per cent in Ringer's solution on vascular tone in the frog. The extended marks indicate the period of change from one solution to the other. *R* = Ringer's solution: *U* = Ringer's solution plus 0.5 per cent urea.

The addition of urea to Ringer's solution perfused through the whole frog has a pronounced effect upon vascular tone as shown in the accompanying record (see Fig. 4). Strengths of solution ranging from .25 per cent to 4.0 per cent were investigated. They invariably produced vaso-dilatation.

The facts reported in this paper point interestingly to the uniformity of reaction exhibited by the musculature of the cardio-vascular system. The substances which relax cardiac tone also relax vascular tone, and the substances which increase cardiac tone likewise increase vascular tone. The unit volume of the heart is increased corresponding to the functional needs of the body. The influences which bring this condition about further aid in fulfilling physiological requirements by producing vascular relaxation; the resultant effect must be a greatly increased velocity of circulation. Apparently therefore, as suggested by Bayliss,[12] there is a complete chemical regulation of the cardio-vascular system which may act independently of the central nervous system.

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[10] JERUSALEM and STARLING: Journal of physiology, 1910, xl, p. 279.

[11] BACKMAN: Skandinavisches Archiv für Physiologie, 1908, xx, p. 5.

SUMMARY.

1. Vascular tone is increased by calcium ions and oxygen.

2. Vascular tone is decreased by sodium and potassium ions, carbon dioxide and urea.

3. The fact is emphasized that the musculatures of the vascular system and of the intestine give opposite responses under the influence of carbon dioxide and oxygen.

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[12] BAYLISS: Ergebnisse der Physiologie, 1906, v, p. 319.

VARIATIONS IN THE RESPONSE OF HEALTHY MEN TO THE DYSPNEIC CONDITIONS PRODUCED BY BREATHING A CONFINED VOLUME OF AIR.

By THEODORE HOUGH.

[*From the Physiological Laboratory of the University of Virginia.*]

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The depth of the respiratory movements in dyspnea 375
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The minute volumes of inspired air 382
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FOR the last two years the writer of this paper has been collecting observations on the response of the respiratory mechanism of adult men (mostly about twenty-one years of age) to the dyspneic conditions produced by breathing a confined volume of air. Special attention has been paid to the respiratory rate, the volume of air breathed per minute, the depth of inspiration, the occurrence of periodic breathing, and to the rôle of inspiratory and expiratory effort by which pulmonary ventilation was secured.

The recording apparatus was the modification of Gad's aeroplethysmograph described in a previous number of this journal.[1] For convenience of reference the diagram of the apparatus is reproduced in Fig. 1. Full details will be found in the text of the previous article. Recently the accumulation of such records has been facilitated by the use of a counter on which the minute volumes are read at the time of the experiment. For this purpose the work adder of the Harvard Apparatus Company has been utilized. A friction pulley, revolving on the same axis as the ratchet wheel, is attached to the truss lever of the aeroplethysmograph near its writing point. It is not necessary to consume space with a description of details, the counter consisting essentially of a ratchet wheel actuated by the recording lever of the aeroplethysmograph, which records at the same time the respiratory movements as in previous experiments. The instrument was calibrated and tested, being found accurate to a small fraction of one per cent.[2]

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[1] Hough: This journal, 1910, xxvi, p. 156.

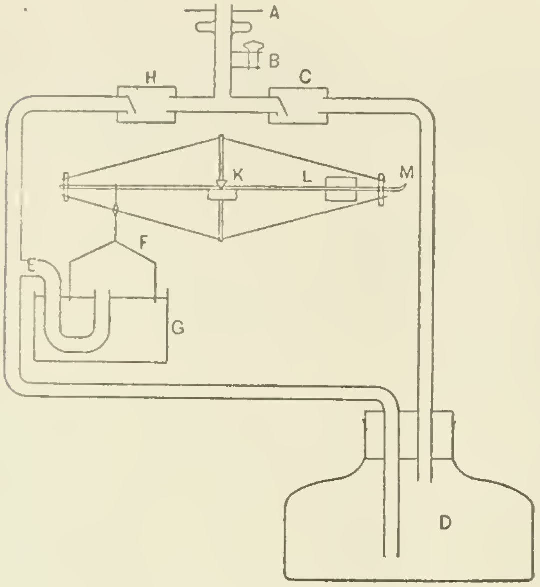

Figure 1. — Diagram of the essential parts of the apparatus. *A*, mouthpiece with lip and teeth flanges; *B*, side tube for taking specimen of alveolar air; *C*, expiratory valve; *D*, 20-litre bottle; *E*, side tube to air chamber, *F*, of the aeroplethysmograph; *G*, the water seal; *H*, inspiratory valve; *K*, knife edge; *L*, counterpoise; *M*, writing point.

Briefly then in all experiments the subject breathed through an air-tight mouthpiece from a confined volume of air which in all experiments was about 30 litres. Valves separated the expired from the inspired air. The carbon dioxid gradually accumulated in the confined space, and the oxygen gradually diminished, although at the end of none of these experiments was the percentage of carbon dioxid in the inspired air greater than 7 nor that of the oxygen less than 14.

To make respiratory tracings of this kind from different individuals strictly comparable, the volume of air used by each person should be proportional to his body weight. The tracings show, as might have been anticipated, that a man of 120 pounds weight lasts longer on the same volume of air than one of 170 pounds. I regret that this point escaped my attention until the experiments were almost completed; but careful tests by two of my students, Mr. Mercer Blanchard and Mr. Charles M. O Connor, show that when the same subject uses 22.5, 30, and 40 litres of air in different experiments, no feature of the tracing seriously changes except the time required to reach a given minute volume or rate. Another series of experiments undertaken for a somewhat different purpose by three other students, Messrs. R. R. Dale, R. L. Kendrick, and Carrington Williams, gives results which indicate the same thing.

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[2] The aeroplethysmograph, valves for separating inspired and expired air, and the counter have all been made in the shop of the Physiological Laboratory of the University of Virginia. While the construction of this apparatus for others must be secondary to the use of the shop for my own laboratory, I am in position, subject to the above limitation, to make the entire apparatus, or any of its parts, at what amounts to the actual cost of production.

The eight tracings reproduced in Figs. 2 and 3 give a fair idea of the observed types of the response of the respiratory mechanism in securing alveolar ventilation during dyspnea. To attain this end of alveolar ventilation two main factors, of course, contribute, namely, the rate and the depth of respiration. The tracings show at a glance how one subject will have recourse chiefly to increased depth (T. H., C. T. P., M. B.), another to increased rate (H. R. E., J. B. S., J. B. L.), while a third will make use of both expedients (S. S. I.). One of the strongest impressions left with the writer from his rather wide experience with these tracings is that the rate and depth of respiration are determined by different physiological mechanisms, which, although closely coördinated and often working together to secure ventilation, yet may work independently of each other. Not only do we find one subject depending almost entirely upon increased rate, another depending almost entirely upon increased depth, and a third using throughout the dyspnea both expedients; but in one case (T. H., Fig. 2), for the first eight or nine minutes of the experiment the increased alveolar ventilation is secured solely by increase of depth, after which recourse is suddenly had to more rapid rate. Essentially the same thing is seen in at least fourteen out of the twenty-five subjects of these experiments, although none of these show so sudden a change of rate. (Compare the tracing from C. T. P. which approaches closest that of T. H. in this respect.)

Review of the literature of respiration shows at least two series of observations which indicate separate mechanisms controlling rate and depth of breathing. The first is the observation of Richet[3] that the hyperpnea produced by exposure to higher temperatures (as when a dog pants) is really a *polypnea* accompanied by decreased depth of breathing. The value of this form of breathing for its purpose is evident, since it increases the output of heat without disturbing the alveolar tension of carbon dioxid.

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[3] Richet: Comptes rendus, 1887, cv, p. 313; Mémoires de la Société de Biologie, 1887, p. 25; Archives de physiologie normale et pathologique, 1888, p. 298.

The other observations to the same effect are those of Scott,[4] who showed that whereas in the normal rabbit dyspnea involves increase both of rate and depth, in vagotomized rabbits there is an increase of depth but no increase of rate. While it is evident from the results given

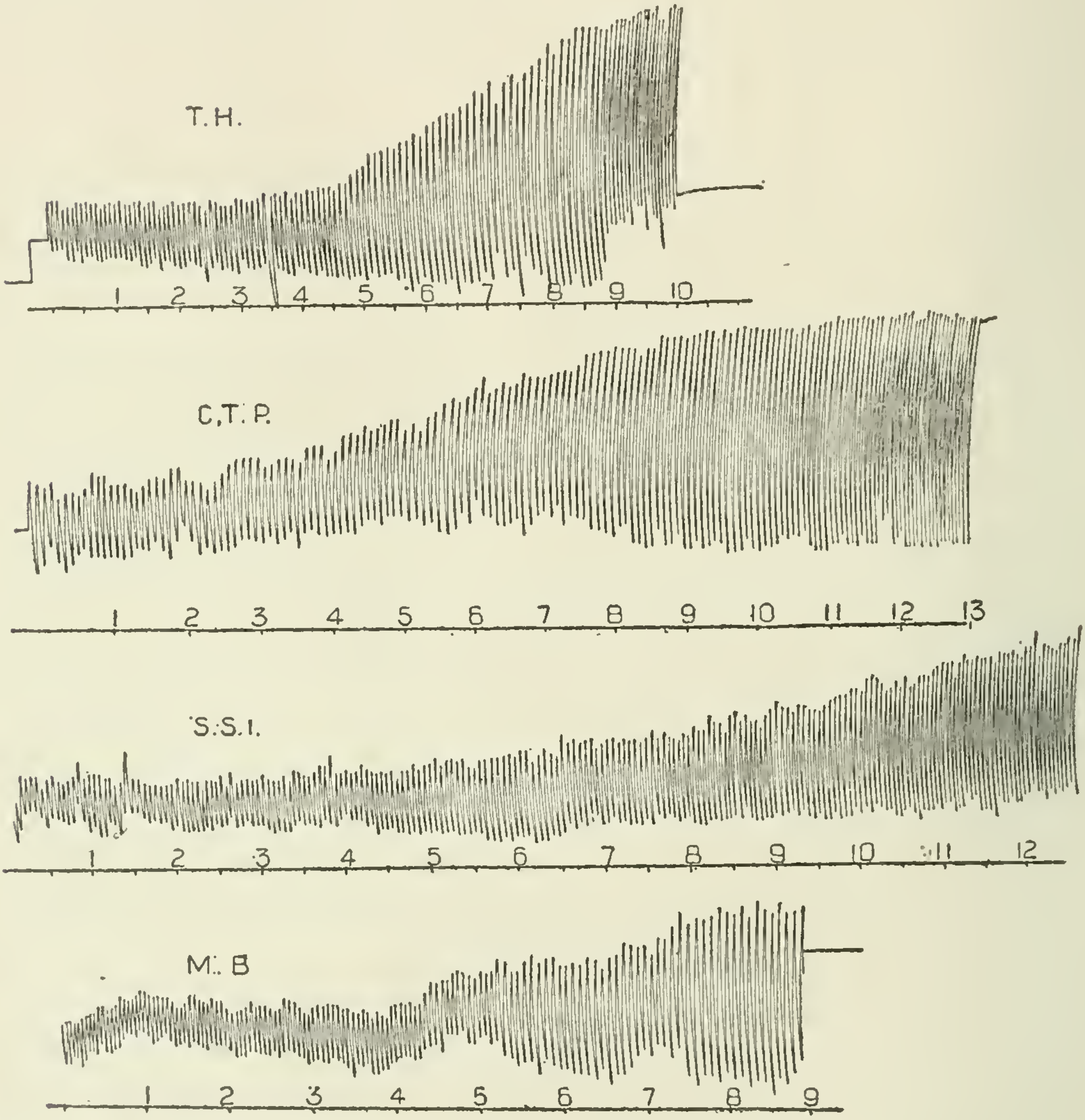

FIGURE 2. — Dyspneic response from four typical subjects of these experiments. In this and the following figure the kymograph drums were not moving at the same speed; but a given rise or fall of the writing point represents in each case virtually the same volume of air inspired or expired, respectively. Upstroke = inspiration. Note especially: T. H.. — Increase of depth until 9th minute; then sudden change of rate. C. T. P. — Increase of depth. Gradual increase of rate after the eighth minute. S. S. I. — Increase of rate and depth from the first. Increase of depth less marked. M. B. — Increase of depth with decrease of rate throughout.

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[4] SCOTT: Journal of physiology 1908, xxxvii, p. 301.

in this paper as well as from those recorded by Haldane and Priestley[5] that increase of the carbon dioxid content of the air does not generally in man produce so quickly an increase in rate as Scott's results would indicate to be the rule in rabbits, the difference may be due to different threshold values for the rate stimulus in the two animals; indeed my own results (*infra*) indicate that there is considerable variation in this respect with normal men. The present point is that

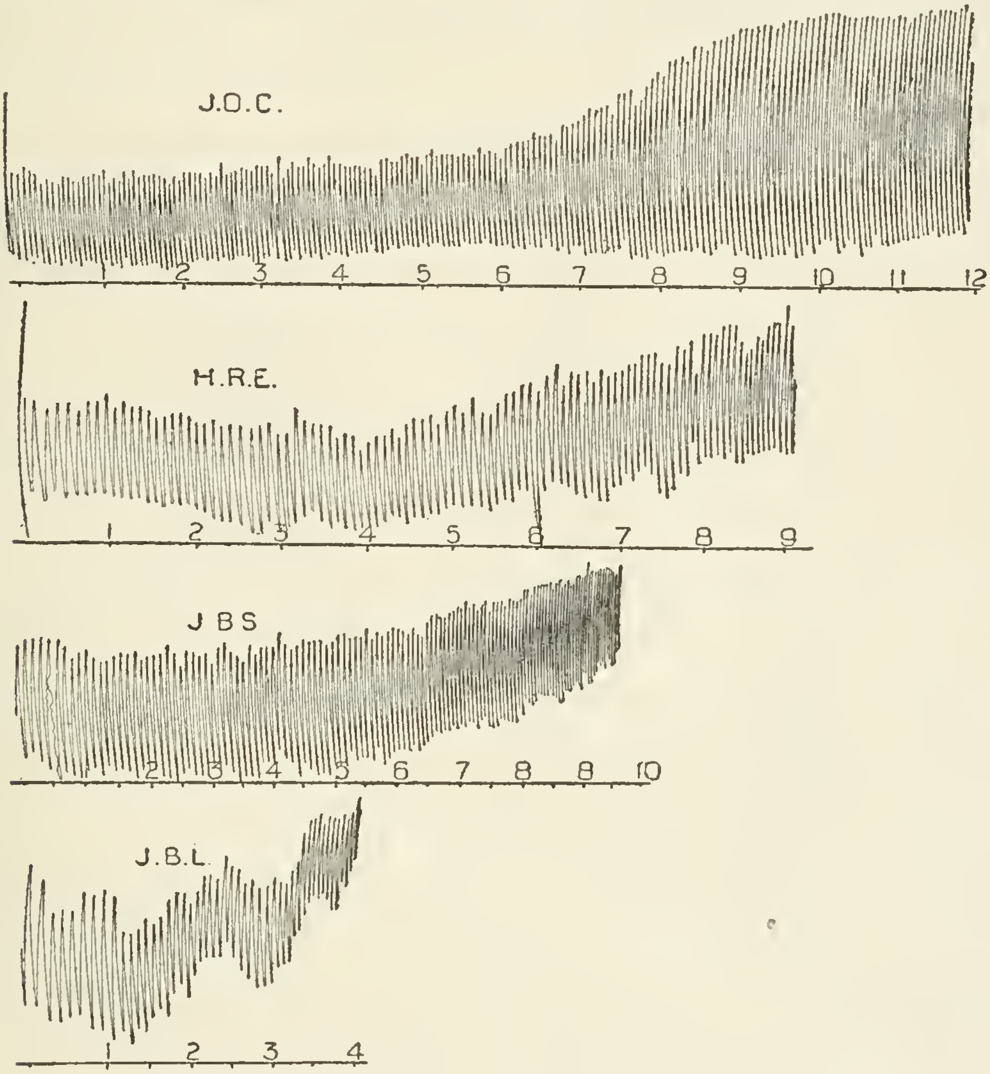

FIGURE 3. — Dyspneic response from four typical subjects of these experiments. Note especially: J. O. C. — No change of rate or depth for six minutes; then only increase of depth. H. R. E. — Little change of depth until the later minutes of the experiment. Increased ventilation secured chiefly by change of rate. J. B. S. — Increase of rate; decrease of depth. J. B. L. — Marked increase of rate with unusual decrease of depth. Experiment ends with tetanus of inspiratory muscles.

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[5] HALDANE and PRIESTLEY: Journal of physiology, 1905, xxxii, p. 225.

Scott's results agree with my own in suggesting different mechanisms regulating rate and depth.

With these preliminary considerations in mind we now pass to a detailed description of the results of our experiments.

I. The Rate of Respiration in Dyspnea.

The changes of rate during the dyspneic response may be classified under five types, best represented graphically by the curves in Fig. 4.

Type 1 occurred in 5 out of 25 tracings.
Type 2 occurred in 3 out of 25 tracings.
Type 3 occurred in 10 out of 25 tracings.
Type 4 occurred in 5 out of 25 tracings.
Type 5 occurred in 1 out of 25 tracings.

The actual respiratory rates per minute from examples of each of these types are as follows:

I. { *a.* — 10, 9.5, 9.5, 10.2, 10.6, 12, 12.8, 14, 14, 14, 14.2, 15, 19.
{ *b.* — 7, 11, 13, 17.

II. { *a.* — 15, 15.5, 15, 15, 15.5, 15, 15, 15, 15, 17.5, 18, 20.
{ *b.* — 7.5, 7, 7, 6.9, 7, 7, 7, 7.8, 7.8, 8.3, 10, 13.

III. { *a.* — 11.3, 11.5, 9.2, 8.3, 7.5, 8.3, 8.2, 8.2, 10.4, 12.7, 13.9.
{ *b.* — 15, 13, 13, 13, 12.6, 11.5, 12, 10, 11, 12, 15, 16.

IV. { *a.* — 9.7, 13.2, 13.6, 12.5, 12.4, 11.1, 11.7, 13, 14.
{ *b.* — 14.5, 15.5, 17, 15.5, 16.2, 15, 15, 14.5, 16.5, 17.5, 19.

V. *a.* — 20, 17.5, 17, 16, 12.5, 12, 10.7, 10.

The rate curves in different experiments on the same individual do not, as a rule, show very marked variations, although there are a few exceptions to this statement. The table given on page 165 of my previous paper represents the usual range of variation. As a rule, a higher rate in the same minute is accompanied, in the same individual, by higher minute volumes.

Attention is called especially to the fact that the rate shows a distinct depression in types 3, 4, and 5, during the period extending from the third to the seventh or eighth minute of the tracing; that is, in 16 out of 25 subjects; also that the rate increases in this period only in type 1, that is, in 5 out of 25 subjects. In the remaining four subjects (type 2) there was no change of rate during this period beyond a slight initial increase or decrease in the first one or two minutes. *Thus out of 25 subjects only 5 showed any increase of rate until after the sixth minute or even later.* The statement commonly found in text books that dyspneic conditions normally increase both rate and depth of breathing is, therefore, misleading and obscures the fact that rate and depth may be controlled by different factors.

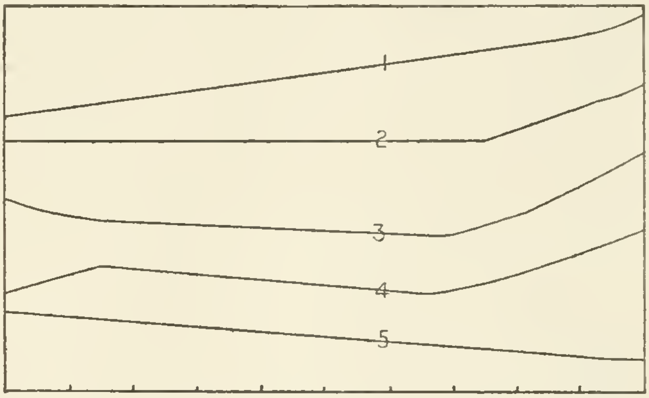

FIGURE 4. — Graphic representation of the types of change in rate during the dyspnea caused by breathing a confined volume of air. The ordinates represent only *changes* in rate; not relative rates as between the different types.

Haldane and Priestley have recorded somewhat the same experience. "It will be seen that the increased alveolar ventilation was at first almost entirely obtained by increased depth of the respirations, there being little or no alteration in their frequency until the alveolar ventilation had been increased to about five times the normal." This statement refers to observations on only 2 subjects. Our results show that while this statement of Haldane and Priestley is not an accurate formulation of a general rule it is a rough approximation to the actual facts observed.

All but one of our subjects increased the rate toward the end of the experiment. The one exception to this rule is shown in the tracing M.B. in Fig. 2. The same reaction was given by this subject in all his experiments.

II. THE DEPTH OF THE RESPIRATORY MOVEMENTS IN DYSPNEA.

Dyspnea is almost invariably ushered in by an increase in the depth of respiration, and in by far the majority of cases, under the conditions of my experiments, this increase of depth continues for at least five or six minutes, that is to say, until the carbon dioxid in the inspired air equals from 4 to 6 per cent. Of our 25 subjects 23 gave an unmistakable initial increase of depth, while only 2 gave decreased depth;

both of these two showed also from the first a steady increase of rate. Indeed it may be stated as a rule to which I find no exceptions in my tracings that early increase of rate is correlated with sub-normal increase of depth (*e. g.*, S. S. I., Fig. 2), or, as in the two cases above mentioned, with actual decrease instead of the usual increase of depth. (See page 377 for consideration of the significance of these points.)

In the later stages of dyspnea there is usually a decrease of depth which is always connected with an increase of rate. When the rate becomes very rapid, the stimulus to the next inspiration becomes effective before the expiratory phase has had time to complete itself. Hence there is usually a more rapid rise of the expiratory base line, an indication of increased tone of the inspiratory muscles.

In one subject an initial decrease of depth lasting for some four minutes was followed by an increase during the rest of the tracing. The same thing was occasionally seen in certain tracings from other subjects, but was not in those cases the constant type for the subject.

In eight subjects the depth of respiration continued to increase until the end of the experiment; or, at any rate, there was no decrease of depth, even when, toward the end of the experiment, the rate had materially increased.

III. THE EXPIRATORY BASE LINE.

By the expiratory base line is meant the line joining the lowest points in the downward or expiratory strokes of the recording lever. In Fig. 5 an attempt is made to represent graphically the types of this line, passing from one extreme in 1 to the other extreme in 4 and 5. It will be seen that in the majority of cases the trend of this line is upward after the first few minutes of the tracing. The initial depression generally seen is due to the heating of the air in the closed space. This factor will exert its influence until the temperature within the system becomes constant, and this usually takes place within four minutes; indeed there is but little change of temperature after the second minute.

If this temperature factor were eliminated, a second factor, acting alone, would cause from the first a gentle and steady rise of the base line. This is the return to the system of a smaller volume of carbon dioxid than of oxygen removed; moreover, this factor must exert its influence in all cases and throughout the experiment, since probably in few of our experiments was the respiratory quotient equal to unity.

The steepness of the rise after the third or fourth minute differs considerably in different tracings showing such a rise. In type 1 it is very rapid (J. B. L., Fig. 3). Type 2 shows likewise a more rapid rise than can be explained by the character of the respiratory quotient. It will be seen that such rapid rises are characteristic of only a small per cent of the subjects of our experiments, although a much larger number show the same character in the last two or three of the ten or twelve minutes during which such experiments ordinarily last.

In type 3 we have approximately the rise which would be caused by the character of the respiratory quotient, and this type comprises 56 per cent of our cases. Here we may assume that the expiratory return of the thorax is complete, but that active expiratory effort does not go beyond this point.

In types 4 and 5, on the other hand, the trend of the base line from about the third to the eighth minute is either horizontal or, more generally, downward instead of upward, and for this the activity of the expiratory muscles must be held responsible, since all other conditions of the experiment must then work toward an upward trend. Eight of our subjects belong in this class, and it is a significant fact that seven of these show a slight decrease from the original rate during this period, while the eighth showed no change from the original rate. Conversely, of those who show a steady rise of the base line (types 1, 2, and 3 of Fig. 5) 80 per cent show either a steady increase of rate or at least no decrease from the original rate. *In general, then, the rate and depth of respiration tend to vary inversely.* It would also seem that where great depth is to be secured, the dyspneic reaction of increased rate is held in abeyance, thus insuring ample time in each respiratory period for the breathing of a sufficient volume of air to give adequate alveolar ventilation.

The question is thus raised whether the depth of respiration determines the rate or *vice versa*. From *a priori* considerations each hypothesis is more or less plausible. Increase of rate may cause decrease of depth by not allowing time for the completion of expiration; the rate in this case would be the cause and the shallow breathing the result. Increase of depth, on the other hand, by securing more perfect alveolar ventilation may delay the adequate stimulus to increase of rate; in this case the deep breathing would be the cause and the slow rate the result. Or it may well be that in different experiments or during different periods of the same experiment either one may be a determining cause of the other. It has been shown that in those cases which show a horizontal or downward trend of the base line we have convincing evidence of distinct expiratory activity, with high efficiency of alveolar ventilation, and in all these cases the rate is kept down or even depressed. Yet, even here, one cannot say whether the low rate is the direct result of the more perfect ventilation, or whether the inadequacy of the stimulus to increased rate merely renders possible more complete expiration by giving sufficient time therefor.

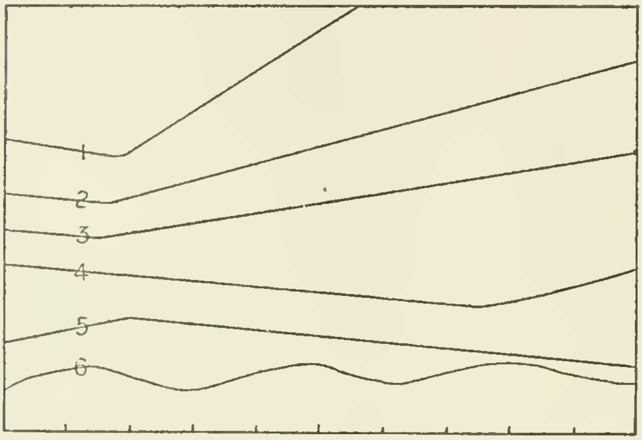

FIGURE 5. — Graphic representation of the types of change of expiratory base line. Type 1 is shown by 1 subject out of 25 (J. B. L.). Type 2 is shown by 1 subject out of 25 (J. B. S.). Type 3 is shown by 14 subjects out of 25. Type 4 is shown by 6 subjects out of 25. Type 5 is shown by 2 subjects out of 25. Type 6 is shown by 1 subject out of 25.

The facts at my disposal do not allow a decision between these two hypotheses; nor should we lose sight of the fact that, logically, we may be dealing with two coöperating causes which mutually influence each other's action; thus active expiration in coöperation with increased inspiration may delay the adequate stimulus to increased rate (*e. g.*, by holding down the alveolar tension of carbon dioxid), while, reciprocally, once the rate stimulus has become adequate and the respiratory period is shortened, more shallow breathing may result from incomplete expiration.

Such indeed, in my opinion, is probably the correct statement of the case. The diminution in depth seen in the tenth minute of the tracing from T. H. (Fig. 2) is undoubtedly caused by the increase of rate which then occurs, for there was no diminution of active expiratory effort at that time. In the case of J. B. L. (Type 1, Fig. 5; tracing J. B. L., Fig. 3) I think the causal factor is more likely an unusual sensitiveness of the respiratory mechanism to the rate stimulus. This subject is unable to continue the experiment longer than three or four minutes at a time. As the tracing shows, the rate rapidly increases, expiration becomes progressively more and more incomplete, and the experiment finally comes to an end, in from two to four minutes with a tetanus of the inspiratory muscles. At this time the carbon dioxid content of the dry alveolar air is over eight per cent and the tension is over 56 mm. Hg. I hope in the near future to make a thorough investigation of this peculiar case. At present I can only say that the onset of inspiratory tetanus is but slightly delayed by the use of deep breathing by the subject. The tendency to increased rate asserts itself even here from the first; soon the pace becomes too fast and the experiment is quickly brought to a close. The cause of this does not, therefore, seem to be the failure to secure the usual alveolar ventilation by the proper use of expiratory muscles, else we should have a much greater prolongation of the experiment when deep breathing is purposely used. On the other hand I have found that the experiment is very greatly shortened by adding to the inspired air about 2 per cent of carbon dioxid, an amount which does not produce respiratory distress in most individuals. In the case of J. B. L. breathing this atmosphere results in a prompt and decided increase of rate (and diminution of depth) which brings the experiment to an end in a minute or less. These results are only preliminary, but they strongly suggest that the cause of the trouble is the premature quickening of the rate, this leading to incomplete expirations and so to imperfect alveolar ventilation.

While, therefore, recognizing the probable importance of the sensitiveness of the respiratory mechanism to the rate stimulus as determining the depth of respiration, I would still urge that equal attention be given to the action of expiratory muscles in securing maximum ventilation of the lungs, as an important factor determining the rate. It is a significant fact that of the eight subjects who show a depression of the expiratory base line (and who therefore seem to make use of active expiratory effort), all but one actually show a decrease from the original rate, and usually this decrease continues from minute to minute until the minimum rate is reached about the sixth to the eighth minute. Subjective impressions, of course, are of little value as evidence in a case like this, but it is worthy of note that the subjective impression is that the expiratory effort holds in check the tendency to begin the next inspiration. Once the expiration is completed, the inspiratory mechanism comes into play as if released from an inhibitory influence. The increase of rate which always comes toward the last of the experiment makes its appearance sooner and is more pronounced in these subjects who do not keep the base line down from the first; and this may be fairly interpreted as meaning that the efficiency of respiration, under the dyspneic conditions of our experiments, is increased by holding in check (whether by direct inhibition or by more perfect ventilation) the tendency toward increase of rate. The interesting question here raised is, what part does the expiratory portion of the respiratory centre play in securing effective alveolar ventilation?

The writer of this paper is convinced that the rôle of the expiratory neuro-muscular mechanism has been too much neglected, and even thinks that it is underrated in the usual teaching of this part of physiology. For example, it is almost universally taught that in ordinary quiet breathing expiration is *wholly* a passive return of the elastic thorax to its normal position; and yet one may safely challenge the least proof of the truth of this statement; indeed we may fairly wonder whether it is supported by anything save the fact that we are not conscious of any resistance to muscular effort during quiet expiration; and yet no one would seriously urge this as a conclusive reason for believing that there is no muscular contraction at all. In favor of the active rôle of the interosseous portion of the *mm. intercostales interni* and the *m. transversus sterni* I can offer the positive evidence of my own experiments[6] upon the respiratory function of the intercostal muscles. In these experiments simultaneous records were taken of the contractions of the diaphragm and of the muscle under investigation, the latter being isolated from the rest of the body, except for a strip containing its artery, vein, and nerve. With the two expiratory muscles above mentioned, some twenty experiments on the dog gave the result that, unless the animal was made apneic by the artificial respiration, these muscles always contracted rhythmically. In other words, *so long as the diaphragm contracted, these expiratory muscles contracted alternately with it.* The contractions were not as great as those of the inspiratory muscles (intercartilaginous portion of the *mm. intercostales interni* and the *mm. intercostales externi*), but there could be no doubt of the fact of contraction. As I wrote then: "Either, then, the animals were always apneic or dyspneic (which is possible) or these muscles act in the normal breathing of the dog," and I am still of the opinion that the balance of evidence is on the side of their contraction during quiet respiration, whether this contraction aids in lowering the ribs or merely makes the intercostal spaces more rigid during the expiratory pressure changes on the two sides of the thoracic wall.

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[6] Hough: Studies from the Biological Laboratory of the Johns Hopkins University, 1892, v, p. 91. See especially p. 96.

The same paper described another striking feature of interest to us in connection with the present discussion, in the behavior of the expiratory muscles during dyspnea, namely, a supplementary contraction which occurs just before the end of expiration and is added on to the contraction which began with expiration. This was especially noticeable in the case of the *m. transversus sterni* but was also seen in the *mm. intercostales interni.* It was as if just before the inspiratory intake of fresh air, the expiratory muscles shorten still more and so render the ventilation secured by the immediately following inspiration more effective.

In the current literature of the subject the neuro-muscular mechanism of lung ventilation is commonly referred to as if it were a single and comparatively simple mechanism, actuated by one or more stimuli (*e. g.*, carbon dioxid or intermediate acids). Even writers who properly insist on attention to alveolar rather than total ventilation do not always seem to take into account the fact that alveolar ventilation itself is the result of the coöperation of several factors each of which may show very different degrees of development or of irritability in different individuals. There seems to be sufficient indication of the existence of three variables in this mechanism, namely:

1. Factors determining the respiratory rate.
2. Factors determining the amount of inspiratory effort.
3. Factors determining the amount of expiratory effort.

The very existence of these possibilities makes it important that in studies upon the respiratory centre — involving, as they so largely do, experiments with dyspneic conditions — the character of the normal response of the subject to such conditions should be known beforehand.

IV. THE MINUTE VOLUMES OF INSPIRED AIR.

Fig. 6 gives the platted curves of minute volumes from seven subjects. These may be regarded as representing the range of variation seen in my experiments. The curve T. H. is typical of that given by 8 subjects; J. B. S., 6 subjects; R. R. D., 4 subjects; and J. O. C. (and J. O. M.), 3 subjects. The curves of J. B. L. and C. L. W. do not coincide closely with any others.

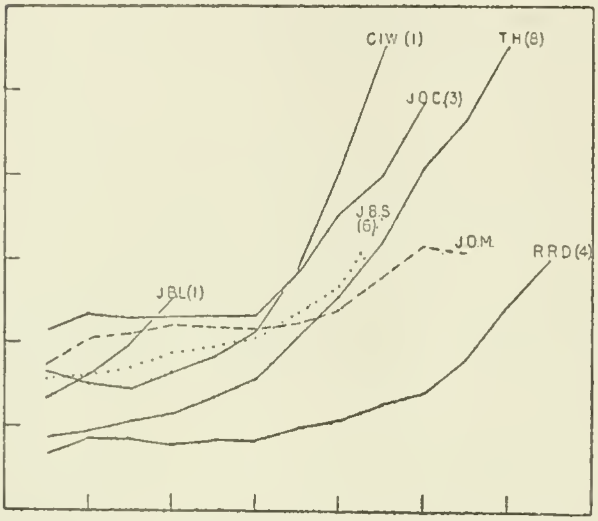

FIGURE 6. — Curves of minute volumes from 7 subjects of these experiments. The numbers in parentheses after the initials are the total number of subjects giving curves of the same type. The curves of J. O. C. and J. O. M. are regarded as belonging to the same type. Abscissæ = 2 min.; ordinates = 10 liters per min.

These curves present differences in the volume of air breathed during the same minute of the experiment, in the rate of increase in successive minutes, and in the form of the curve. We may begin with a discussion of the first two of these points of difference. There can be no doubt that these are partly due to the use of the same volume of air by subjects of different body weight. This is the case with R. R. D., whose minute volume per unit of body weight is about the same as that of T. H., and the slower increase of minute volume is to be similarly explained, since, on 20 litres of air, he gave a curve closely approximating that of T. H. (see also page 370 of this paper).

This is not, however, the sole determining factor. C. L. W., J. B. S., and T. H. are of about the same body weight and general build, and yet the amount of air respired by them in a given time is very different. During the first five or six minutes, C. L. W. and J. B. S. each respire some 7 or 8 litres per minute more than T. H. The same thing is even more strikingly shown by the curve of J. O. M. This subject's weight is about 75 per cent of that of the other three, and yet until the sixth minute he breathes more air than any of them, and almost twice as much as T. H. Determination of the normal resting alveolar tension throws no light on the differences, for all give from 38 to 40 mm. Hg for carbon dioxid. What these tensions are during the course of the experiment has not been determined. Remembering that our figures represent *total* and not *alveolar* ventilation, we at once suspect that the great differences in pulmonary ventilation may be due to differences in the volume of the dead space or in the rate of breathing, or to both combined. To take the extremes, a subject with relatively large dead space whose breathing is of the rapid, shallow type would evidently have to respire more air per minute than one with relatively small dead space whose breathing is deep and slow. As yet our information as to the variations in the size of the dead space is somewhat limited. Loewy[7] estimated it as 140 c.c. with upper and lower limits of 150 c.c. and 100 c.c. Haldane and Priestley (*loc. cit.*) found in two subjects averages of 142 c.c. and 189 c.c. for each, with variations between 90 c.c. and 186 c.c. for one subject and 124 c.c. and 243 c.c. for the other. The wide range of variation in determinations from the same subject indicates either very great inaccuracy of method or else variations from time to time in the size of the bronchial tree due to the action of its musculature not suggested in current writing on the subject. Since, however, the curves of C. L. W., J. B. S., and T. H. are in each case the averages of at least six experiments which do not differ greatly from each other, we may assume that the question is not seriously complicated by such variations from time to time of the size of the same bronchial tree. Is it possible then to explain the differences of total ventilation on the basis of the size of the average dead space, the rate, and the depth of breathing? The rates of J. B. S. and T. H. differ but little, and reference to their tracings (Figs. 2 and 3) will show that the difference of their total ventilation is due wholly to the depth of the individual respirations. Is this greater depth made necessary by the larger dead space? It is easy to show that such an assumption fails to give a complete explanation; thus in the fourth minute J. B. S. breathed 7 litres of air more than T. H. Since J. B. S.'s rate at that time was 11.3 respirations per minute, the greater amount of air breathed can be explained as due to the larger bronchial tree only by making the impossible assumption that J. B. S.'s dead space is 620 c.c. (7000 ÷ 11.3) larger than that of T. H. Or we may make the following instructive calculation in comparing the cases of C. L. W. and T. H. Let us make the very improbable assumption that the dead space of C. L. W. is 250 c.c. and that of T. H. 100 c.c. (the two extremes of the observed recorded values). Multiplying these by the respective rates for the fourth minute, there would be used of the total minute volumes (16.4 litres and 11.4 litres) in the two cases 3.95 litres and 1.0 litres to fill the dead space; *i. e.*, a difference of 2.95 litres against an observed difference of 5 litres. A similar comparison of the cases of J. O. M. and T. H. gives 3.75 litres and 1.0 litres to fill the dead space, or a difference of 2.75 litres as against an observed difference of 10.6 litres.

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[7] Loewy: Archiv für die gesammte Physiologie, 1894, lviii, p. 416.

These assumed differences in the size of the dead space are unquestionably the maximal extremes possible and are improbable in the highest degree. We must, therefore, conclude that the combined factors of size of dead space and character (rate and depth) of breathing fail, and at times signally fail, to explain the observed differences in the minute volumes of air breathed. If we assume more probable limits of variation in the size of dead space, we find that not more than 10 to 15 per cent of the higher minute volume of J. B. S., C. L. W., J. O. C., and J. O. M. can be explained in this way; something else is responsible for the greater part of the difference, and I think there are two remaining possibilities. Either they represent differences of carbon dioxid production or else something during the first five or six minutes causes an over-ventilation of the lungs. I am now testing the actual rôle of this second possibility by determinations of the alveolar tensions of carbon dioxid and oxygen during the dyspneic reaction in several of the subjects of these experiments. With regard to the first possibility, the observations made on the range of variation in the liberation of energy per kilogram of body weight in adult men give the upper limit in different series of determinations as 38, 33, and 56 per cent higher than the lower limits, which of course indicates that specific individual respiratory exchange of the tissues may be responsible for a large amount of the variation of total pulmonary ventilation seen in our cases.[8]

We may therefore conclude that the great differences of pulmonary ventilation in the subjects of our experiments are due only in small part to the size of the dead space or even to a combination of this factor with a rapid, shallow type of breathing, since this character of breathing is absent in three out of the four subjects and is but slightly marked in the fourth subject; by far the most important factors are what we may term the specific respiratory exchange of the tissues and, to an undetermined extent, a possible tendency on the part of some individuals to over-ventilation of the lungs in the earlier minutes of a dyspneic tracing.

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[8] See TIGERSTEDT: NAGEL's Handbuch der Physiologie, i, p. 544.

We now pass to the consideration of the *form of the curve* of minute volumes in our experiments. Mathematical calculation shows that in order to maintain a constant alveolar tension of carbon dioxid when the production of this gas by the body is constant while the amount in the inspired air increases directly as the time, the volumes of alveolar ventilation in successive units of time must make an asymptotic curve. Suppose, for example, the subject is producing any fixed amount of carbon dioxid, let us say 240 c.c. per minute. With an alveolar tidal air volume of 400 c.c. containing 5 per cent of carbon dioxid, 20 c.c. of this gas would be removed with each breath, and 12 respirations would be necessary each minute to effect perfect alveolar ventilation. If now the inspired air contains 1 per cent of carbon dioxid, 400 c.c. of alveolar tidal air would now remove only 4 per cent of 400 c.c. = 16 c.c. and 15 × 400 c.c. of ventilation would be required to remove the 240 c.c. Two per cent of carbon dioxid in the inspired air would similarly require 20 × 400 c.c., and 3 per cent would require 30 × 400 c.c. We would thus have as the proportional ventilation for successive minutes:

Time units	0	1	2	3
Ventilation	12	15	20	30

Of course the respiratory movements of the body do not, under the conditions of our experiments, effect the removal of the carbon dioxid as fast as it is produced, since we know that the alveolar tension of this gas increases; but Haldane and Priestley have shown that the increased ventilation during dyspnea does actually keep down to a remarkable extent the alveolar tension of carbon dioxid, so that there should be a fair approximation to the curve we have outlined above.

Actual analysis of the inspired air shows that at least for the first six or seven minutes of our experiments the oxygen decreases and the carbon dioxid increases in direct proportion to the time; and the curves of total pulmonary ventilation in Fig. 5 show that these are usually of the asymptotic type called for by the *a priori* considerations already given. Twenty out of 25 of our subjects unmistakably reacted in this way; 2 gave curves too irregular for classification; there remain for consideration three curves which in the first six minutes of the experiment, at least, apparently depart from the asymptotic type. These are the curves of J. O. C., J. O. M., and a third subject whose curve rather closely corresponds to that of J. O. C.

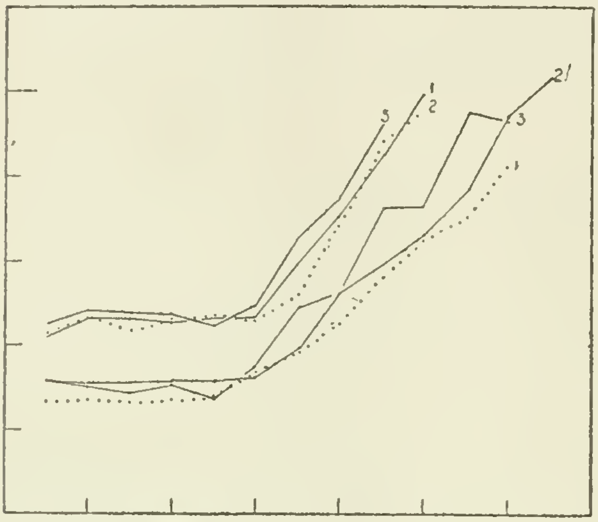

FIGURE 7. — Minute volume curves from six experiments from J. O. C. The three with higher minute volumes were from the series of 1910, and the three with lower volumes from the series of 1911. Ordinates and abscissae as in Fig. 6.

These aberrant tracings are at present the subject of investigation, and we shall limit ourselves in the present paper to a description of their most striking characteristics as observed in the case of J. O. C., upon whom a sufficiently large number of experiments have been made at different times to make sure that we are dealing with a constant type of dyspneic response. Fig. 7 gives six minute-volume curves from this subject, three taken during February and March, 1910, and three during March, 1911. The three curves from the former period are selected as typical from a total of eight obtained at the same time. It will be seen at once that the minute volumes in the second series are lower than in the first, although there is no change in the frequency of respiration; the greater ventilation of the lungs is caused entirely by the smaller depth of respiration, another example of the independence of the two methods of regulating the breathing. From the first to the second minute there may be a slight rise of the curve, a feature of a number of other tracings, which are otherwise of the usual form. It is probably due to other than strictly dyspneic causes. It is seen in the first series from J. O. C., but not in the second. With this exception, *there is no change whatever in the volume of air breathed until after the sixth minute in the first series and until after the fifth minute in the* *second series.* In this time T. H. has almost doubled his minute volume, R. R. D. (on his proportional air supply of 20 litres) has increased his by more than 60 per cent, and 15 other subjects of experiment show increases between these limits.

This is the strikingly aberrant feature of the curves from these three subjects. While the number of tracings from J. O. C. is greater (and hence we have based the above description on them), there are three each from J. O. M. and H. H. V., which, although not so concordant as to volume of air breathed, nevertheless show in each tracing the absence of any increase of ventilation until a comparatively late period of the experiment.

It is also noteworthy that of all our subjects these three show the highest minute volumes for the first five minutes of the experiments. This is especially striking in the case of J. O. M., whose weight is 45 per cent less than that of H. H. V. and J. O. C. Over-ventilation of the lungs during the first part of the tracing is at once suggested as an explanation. As already stated, this possibility is at present the subject of investigation. I can only say that there were no subjective signs of acapnia in any case. It is also hardly to be expected, on this theory, that the stimulus causing such over-ventilation should be so perfectly correlated with the dyspneic stimulus to increase of ventilation as to bring about the result of virtually perfect uniformity of minute volumes for five or six minutes.

Once the pulmonary ventilation begins to increase in these three subjects, it proceeds according to a curve quite normal for men of the same weight and on the same volume of air; that is, J. O. C. and H. H. V., men of heavy build, increase rapidly, while J. O. M. increases at about the same rate as R. R. D., who is of his general build.

The importance of attention to aberrant tracings like these is obvious. Unless their peculiarities can be explained as due to other than dyspneic causes, they suggest that during dyspnea other causes than the carbon dioxid tension of the blood take some part in the regulation of respiration.

We may conclude for the present that 20 out of 25 subjects give curves of minute volumes which seem to accord with the theory that increase of pulmonary ventilation during dyspnea is entirely the result of increase of the carbon dioxid tension of the blood flowing through the respiratory centre; on the other hand 3 subjects seem to give an aberrant type of response which suggests the possibility of another factor regulating the breathing movements.

V. Additional Observations.

Some minor points may be added. The minute volumes during the first two minutes of the experiments varied as follows:

8–10 litres	11 subjects.
11–13 litres	9 subjects.
14–18 litres	4 subjects.
20 litres	1 subject.

Attention has already been called to the fact that of the 5 subjects with minute volumes above 14 litres 3 belong to the aberrant class described in the last section. The other 2 gave good asymptotic curves throughout.

Attention was also paid to the occurrence of periodicity in the breathing. This is usually present in slight degree. The rather unexpected result was that it is more pronounced during the first six minutes than afterwards. Thus 23 subjects showed some degree of periodicity during the first period and in 13 of these it was very distinct. Sixteen showed some degree of periodicity during the second period (*i. e.*, after the sixth minute) and in only 3 of these was it pronounced.

It often happens that the asymptotic form of volume curve is departed from for a minute or more of the experiment, and this occurs very frequently in the last minute of a prolonged experiment, when the working capacity of the respiratory mechanism seems to be unequal to the task of effecting the full ventilation of the lungs. (See Curve 3 of the 1911 series, Fig. 7.)

The respirations in the first two minutes were deep (1200 c.c. or more) in 9 subjects and shallow (600–800 c.c.) in 9. In the remaining 7 subjects the breathing was neither of the distinctly deep nor of the more shallow type. Each individual usually showed the same type in this respect. No clear correlation can be traced between the type of normal respiration in this respect and the ability to withstand dyspneic conditions.

SUMMARY.

1. This paper describes the various types of response of the respiratory mechanisms of 25 adult men to the dyspneic conditions produced by breathing a confined volume (30 litres) of air. The carbon dioxid of the respired air increased and the oxygen diminished, at least for six or seven minutes of the experiment, directly proportionally to the time.

2. To make experiments of this kind strictly comparable, the volume of air used by each person should be proportional to his body weight.

3. Tracings are given which show considerable variation in the character of the dyspneic response of different individuals, although the response of the same individual at different times is fairly uniform. These variations seem to be due to the different extent to which different persons use the three factors of frequency, inspiratory effort, and expiratory effort to secure increased ventilation of the lungs.

4. These three factors of pulmonary ventilation, while coördinated, still seem capable of more or less independent action. Hence one subject may not change the rate, while another does not change the depth. Uusually both factors are utilized, although not, as a general thing, to the same extent at the same time, the more common method being to begin by increasing the depth and later to have recourse to increase of rate.

5. The rate and depth of respiration tend to vary inversely, marked increases of rate being accompanied by little or no increase of depth and occasionally by decrease of depth; increase of depth, on the other hand, is usually accompanied by decrease of rate. This relation between depth and rate, which, as shown by Haldane and Priestley, obtains during normal quiet breathing, is equally true of dyspneic breathing.

6. Only 20 per cent of our subjects showed increase of rate during the first six minutes of the experiment. Of the remaining 80 per cent, 12 showed virtually no change during this same period, while 68 per cent showed a decrease of rate during this period. In the later minutes of the experiment increase of rate is the rule, although one subject (M. B., Fig. 2) continued to decrease the rate as long as he was able to continue the experiment.

7. Twenty-three out of 25 subjects increased the depth from the first. This increase of depth was most marked in those cases which showed decrease of rate. The decrease of depth generally seen toward the last seems to be due to the fact that the rapid rate does not allow time for the completion of the expiratory effort.

8. Attention is called to the probable rôle of the expiratory muscles in securing proper ventilation of the lungs. Reasons are given for thinking that the use made of these muscles differs in different people (Section III). Where expiratory effort is greatly increased, there is always a concomitant decrease of rate, this form of breathing being apparently the most effective in moderate degrees of dyspnea. Reasons are also given for thinking that even in normal quiet breathing expiration is not a passive act, but usually involves contraction of expiratory muscles.

9. The pulmonary ventilation in the dyspneic response to regularly increasing content of carbon dioxid in the inspired air usually increases in the form of an asymptotic curve. This is apparently in harmony with the theory that the chief stimulus regulating dyspneic pulmonary ventilation is the tension of carbon dioxid in the blood. An aberrant type, however, is described in which there is no increase of ventilation for the first five or six minutes of our experiments, after which time the curve assumes the usual asymptotic form. These peculiar curves are at present the subject of investigation.

10. Some subjects show a remarkably large pulmonary ventilation in the first minutes of the experiment. This is due only to a small degree to differences of the size of dead space or to a rapid, shallow type of breathing. It would seem that it must be explained either by the greater specific intensity of the respiratory processes in the tissues, or else by a tendency to over-ventilation of the lungs. This also is at present the subject of investigation.

While I am indebted to all my students who have served as subjects of these experiments, I am under special obligations to Dr. John B. Setzler and Dr. Charles L. Williams, both of whom have aided me by a large number of experiments which they have made upon themselves.

THE CONTROL OF PANCREATIC DIABETES IN PREGNANCY BY THE PASSAGE OF THE INTERNAL SECRETION OF THE PANCREAS OF THE FETUS TO THE BLOOD OF THE MOTHER.

By A. J. CARLSON and F. M. DRENNAN.

[*From the Hull Physiological Laboratory of the University of Chicago.*]

THE first experiment of this series was made during November, 1910. In the operation of pancreas removal in the course of another investigation, it was found that the dog was pregnant. After the operation it was noticed that the rise of the sugar content in the urine was slower and the highest percentage was less than in normal dogs after pancreatectomy. According to Forschbach's experiments on uniting two dogs with subsequent removal of the pancreas of one it was found that neither of the dogs developed diabetes,[1] thus showing that the internal secretion of the dog with the pancreas passed through the cross circulation to the pancreatectomized animal and prevented the diabetes. This, together with our experiment, led us to believe that the pancreas of the fetus might furnish sufficient internal secretion, which passing through the uterine membranes into the maternal blood would influence if not prevent the diabetes in the mother after pancreatectomy.

After pancreatectomy in normal animals we have found, as have other investigators, that sugar invariably appears in the urine within eight to sixteen hours, and reaches its highest point — seven to ten per cent — within twenty-four to thirty-six hours after the operation.

Dog 1. — In our first dog it was noticed, as mentioned, that the sugar was not found in the urine as soon after the operation nor did it reach as high a level. The animal died, by accident, on the sixth day after the operation. On post-mortem examination the pups were found to be about one and one-half inches long, hence likely within the first half of term, as the mother was a rather large dog, weighing nine kilos.

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[1] Forschbach: Archiv für experimentelle Pathologie und Pharmakologie, 1908, ix, p. 131.

Dog 2. — In our second dog the pups were less than an inch long and the course of the diabetes was practically the same as in a normal animal. It was, to say the least, not augmented in any way.

Dog 3. — A third dog, operated on May 10, 1911, in which the fetuses, judging from their size, were within three weeks of full term, lived three days without showing a trace of sugar in the urine. Six samples were collected before the death of the animal, and a test was made on the urine found in the bladder at death. On post-mortem examination the pups were found dead. The viscera were in good condition.

Dog 4. — On May 15th we performed a pancreatectomy on a pregnant bitch within three weeks of term. The animal lived two days, giving four samples of urine, none of which showed any sugar. The urine in the bladder at death was also tested, but showed no sugar.

Dog 5. — On May 29th the pancreas was removed from a pregnant bitch nearing full term. On May 31st, forty-eight hours after the operation, the animal was put under ether and the abdomen opened. The pups, seven in number, were all found to be alive and in seemingly good condition. They weighed 140 gm. each, the mother weighing 7.2 kgm. at the time of operation. Three samples of urine were collected during the forty-eight hours after the operation and a sample taken from the bladder at death, none of which showed even a trace of sugar.

Dog. 6. — On June 11th pancreatectomy was performed on a pregnant bitch nearing full term. The operation was completed in about forty-five minutes, so that the animal was under the influence of ether as short a time as possible, as it appears that in the later stages of pregnancy prolonged ether anesthesia has more deleterious effects than on non-pregnant animals. Recovery was good, and the animal continued in good condition and was observed for five days without showing a trace of sugar in the urine. During this time she was given 100 c.c. of milk twice per day, with all the water she wanted to drink. Polyuria was not marked and the wound in the abdomen did not ulcerate so much as is the case in normal dogs after pancreatectomy. The animal appeared to grow weak, however, so that it seemed doubtful whether she would be capable of giving birth to the pups even if allowed to run to full term; and as it was highly desirable to demonstrate that glycosuria did develop in this animal after loss of the fetuses it was decided to perform a Cesarean section. On June 16th the two horns of the uterus were extirpated and found to contain six pups weighing 127 gm. each. The mother weighed 5.4 kgm. Recovery was good, and no excessive temperature was noticed after the operation. The table shows the sugar content of the urine on the succeeding days:

Date	June 17		June 18		June 19
Time	7 A. M.	2 P. M.	Sample lost	2 P. M.	8 A. M.
Amount of urine in c.c.	50	40	. . . .	70	50
Per cent sugar . . .	0.93	3.1	. . . .	1.77	1.10

June 19th the experiment was discontinued and the animal killed with ether. A study of the table will show that after removal of the fetuses the typical curve of experimental diabetes after pancreatectomy was found — sugar appearing in the urine within fourteen hours — reaching its highest point within twenty-four hours and then gradually coming back to a fairly constant level. The height of the curve was not so great as that found in normal animals after pancreas removal, for in normal animals the great rise is probably due in part to a flushing out of the stored glycogen of the liver, and this would not be so great in an animal operated on six days previous and kept on a diet of 200 c.c. of milk per day in addition to the condition of advanced pregnancy.

From these experiments it seems clear that in a normally developed fetus nearing full term the pancreas is so developed, qualitatively and quantitatively, as regards the internal secretion necessary for normal sugar metabolism that the fetal pancreas acts as a residual pancreas of the mother on complete pancreatectomy of the latter. The internal secretion of the fetal pancreas passes through the uterine membranes in sufficient quantity to prevent diabetes in the mother. At just what stage of pregnancy the fetal pancreas is able, qualitatively and quantitatively, to take up this function we are not as yet able to state, but further work is being done in this laboratory to determine this point. So far as we are aware there is no diabetes in human infants born prematurely in the fifth to the seventh month of gestation. This may be due to the fetal tissues having the power to maintain the carbohydrate metabolism normal even in the absence of the internal secretion of the pancreas, a condition that appears to exist in the pig even in adult life; or, in the second half of gestation, the Islands of Langerhans are capable of normal functioning. Our present experiments support the latter hypothesis.

There is a seeming discrepancy between our present results on pregnant dogs and the usual clinical experience on the effects of pregnancy on the course of diabetes in the human. The clinical experience appears to be unanimous on the point that pregnancy augments the diabetic symptoms, and hence the practice to terminate the gestation in diabetic mothers. Now, even if in its primary cause diabetes mellitus were identical with that of experimental pancreatic diabetes, the favorable action of the fetal pancreas on the mother would come late in pregnancy, and the disturbances in digestion, circulation, and emotional states, etc. of the first half of pregnancy would undoubtedly act unfavorably. But so far as we have been able to learn the unfavorable action of pregnancy in clinical diabetes during the second half of gestation is even greater than during the first half. If this is true, it would seem to indicate a primary difference in the etiology of diabetes in man and of experimental pancreatic diabetes in other mammals. The difference may be only apparent, however. If the diabetes in the mother is caused by the depression of the pancreas by some substance in the blood, or by the inhibition or neutralization of pancreatic secretion by substances in the blood, these substances would in all probability act in the same way on the fetal pancreas or pancreatic secretion, thus giving the usual clinical findings. Unless such cases are already in the clinical literature, it would seem highly important to determine whether a child born of a mother in the condition of diabetes mellitus and running the usual course of increase in the severity of the disease during gestation, maintains a normal sugar metabolism. If it does it would seem that the pancreas or the pancreatic secretion (internal) is not primarily involved in the diabetes of the mother, or that the pancreas or the pancreatic secretions (internal) are depressed or neutralized by toxic substances in the blood of the mother, and that these substances act in the same way on the fetal pancreas or pancreatic secretions. The orientation thus given would enable one to attack some of the problems of diabetes by the methods of blood transfusion.

THE PRESENCE OF THE INTERNAL SECRETION OF THE PANCREAS IN THE BLOOD.

By FRED M. DRENNAN.

[*From the Hull Physiological Laboratory of the University of Chicago.*]

THIS work was undertaken on the suggestion and under the direction of Dr. Carlson in the fall of 1910. It was begun on the hypothesis that there is an internal secretion from the pancreas which has a direct regulating power on the carbohydrate metabolism of the animal, and that the most probable place for this secretion to be found is in the blood of the animal. Forschbach,[1] in his experiments on uniting two animals, found that after cross circulation had been established as shown by injecting iodine into one animal and finding it eliminated in the urine of the other, removal of the pancreas of one was not followed by diabetes of either animal. He also tried some transfusion experiments in which he found that the percentage content of sugar in the urine of a diabetic animal was lessened by transfusion from a normal animal. Minkowski[2] also performed transfusion experiments with similar findings. These results could be explained by a dilution hypothesis — that is, all the blood of the diabetic animal with its high sugar content was drawn off and replaced by blood of a normal dog containing the normal percentage of sugar; hence, of course, the sugar content in the transfused animal would be less, and there would be less elimination in the urine. It seemed that if by injection of normal defibrinated blood without previous bleeding, we could get a fall in the sugar percentage, the fact of an internal secretion would be more conclusively proven. Alexander and Ehrmann,[3] and De Domenicis[4] made similar attempts but with negative results. However, the method seemed so feasible, and the results, if secured, so conclusive, that the research was attempted.

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[1] Forschbach: Archiv für experimentelle Pathologie und Pharmakologie, 1908, lx, p. 131.

[2] Minkowski: *Ibid.*, p. 395.

[3] Alexander and Ehrmann: Zeitschrift für experimentelle Pathologie, 1908, v, p. 367.

[4] De Domenicis: Gazette Int. Medicine, 1910, xxxvii, p. 435.

The method used was to perform a complete pancreatectomy and then make blood injections at different times in the course of the diabetes which followed. The sugar tests were made with Fehling's and Benedict's solutions. There may be some criticism in regard to titration methods of quantitative analyses, but the changes observed in these experiments were great enough so that titration methods were considered exact enough when run with the proper number of controls.

According to Lusk's[5] results the amount of sugar eliminated is directly influenced by the intake of carbohydrate and less directly by the intake of protein and fatty food, so in order to obviate these changes the animals all through the series were fed only 100 c.c. of milk twice per day. This had the advantage that it prevented or diminished the diarrhœa following pancreatectomy in addition to the fact that it gave the dog a constant diet.

The blood for injections was always drawn under sterile conditions into a sterile flask through sterile tubes and defibrinated. Then it was injected into the leg vein of the diabetic animals. Uniform-sized injections, 150 c.c., were used in all cases, since the animals used were all of practically the same size — seven to eight kilos.

RESULTS.

It was found, as it had been by Von Mering and Minkowski[6] and other investigators, that the course of the diabetes was fairly constant. Sugar invariably appeared in the urine from six to eighteen hours after complete pancreatectomy, reached its highest level, seven to ten per cent, within twenty-four to forty-eight hours — then came gradually back to a fairly constant level of about four per cent. The following table will show the course of a typical case: —

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[5] LUSK: Journal of the American Medical Association, 1910, lv, p. 2105.

[6] VON MERING and MINKOWSKI: Archiv für experimentelle Pathologie und Pharmakologie, 1890, xxvi, p. 371.

TABLE I.

DOG I. — COMPLETE PANCREATECTOMY. — DECEMBER 30, 10 A. M.

Date.	Time.	Amt. Urine.	Sugar.	Sugar.
		c.c.	per cent.	gm.
Dec. 30	3.30 P. M.	165	2.11	3.48
Dec. 31	7 A. M.	140	2.74	3.836
	1 P. M.	115	4.90	5.635
	7 P. M.	190	7.69	14.611
Jan. 1	7 A. M.	225	5.219	11.742
	1 P. M.	195	4.50	8.775
Jan. 2	7 A. M.	120	3.39	4.788

Besides the typical sugar findings, failure of the wounds to heal, infection from any skin injury, stitch ulcers, very rapid loss of weight, — one third the body weight in two weeks — excessive thirst, polyuria, progressive weakness and death from complications or death in coma were the usual findings, as have been noted by other workers on experimental pancreatic diabetes.

It was found that intravenous injections of 150 c.c. of normal blood drawn as described above, always caused a fall in the percentage of sugar in the urine and in the total quantity eliminated for the twenty-four hours following the injection. A few typical results follow in Table II, selected from experiments on twelve dogs, with a total of twenty-two injections of normal blood: Dog I, three injections; Dog V and Dog X, one injection each.

In the case of Dogs XI and V (*a*), Table II, it will be noticed that the injections were made during the primary rise of the sugar curve. It will also be noticed that the fall in percentage composition of sugar was greater than when the diabetes had been of longer standing, so that the curve had reached its level, as it had in Dogs I, V (*b*), and X. These results are selected from six experiments, all of which show almost identical results.

One of the criticisms which may be brought up concerning the work is that, as in the transfusion method, the results might be due simply to dilution of the sugar present in the diabetic animal, as it is seen that the fall comes soon after the injection and lasts only through a period of twenty-four hours or less. But it will be remembered that after pancreatectomy the height of the sugar curve is reached in twenty-four to forty-eight hours with the sugar appearing in from six to eight hours. We, then, would expect that the internal secretion found in 150 c.c. of injected blood would influence the sugar metabolism for a shorter time than that found in the blood and lymph of the animal at the time of pancreas removal, hence the result should pass off within twenty-four hours' time. Again, if the dilution criticism should hold, we would expect the least fall when the sugar content is largest, that is, when the injection is made during the primary rise, but it was found that the greatest fall resulted from these injections. However, with this point in mind, the following experiments were performed:

1. Under comparable conditions 100 c.c. of physiological salt solution were injected into a diabetic animal. This was considered to be about the amount of fluid present in the 150 c.c. blood injections. Dog XI (*b*), Table II, shows a typical result. It is seen that there is practically no change in the percentage of sugar nor in the total amount eliminated for the twenty-four hours following the injection, thus indicating that dilution is not the causative factor.

2. Under comparable conditions, again, 150 c.c. of defibrinated blood drawn from pancreatectomized dogs, hence blood which contained no internal secretion, was injected into diabetic dogs. Two of the four experiments carried out are given in Table II, Dogs XII and V (*c*). It will be seen that the curve runs along as though no injection had been made. This experiment is, of course, open to criticism in that the blood injected had practically the same sugar content as that of the animal tested. It had been noticed that stale blood did not give the typical results; hence to answer the above objection the following experiments were tried:

3. Blood was drawn from a normal animal under sterile conditions, defibrinated, and placed in the ice box for twelve to twenty-four hours. It was then brought to body temperature and injected into diabetic animals with the results shown in Table II, Dogs VIII and XII. These experiments seem especially important. (*a*) They check up conclusively on the dilution hypothesis, for in every particular save that the blood stood for a few hours, the injections are identical

TABLE II.

	Date.	Time.	Amt. Urine.	Sugar.	Sugar.	Totals.
				per cent.	gm.	
Dog I[1]	Jan. 25–26[2]	10.30 P. M.	345	4.20	14.49	
		7 A. M.	270	5.181	13.9887	
		7.30 A. M.	125	4.273	5.341	
		2 A. M.	125	4.20	5.25	39.0697
	Jan. 26–27	10 P. M.	400	2.958	11.832	
		7 A. M.	310	1.726	5.25	
		7.30 A. M.	115	2.958	3.4017	
		2 P. M.	200	3.164	6.328	26.8117
	Jan. 27–28	10 P. M.	380	4.717	17.5246	
		7 A. M.	495	5.435	26.903	
		2 P. M.	150	4.30	6.45	50.877
	Jan. 29–30	10.30 P. M.	365	5.494	20.053	
		7 A. M.	425	5.102	21.6835	
		2 A. M.	310	4.808	15.9048	57.6413
	Jan. 30–31[3]	4 P. M.	165	6.849	11.3067	
		8 P. M.	115	1.908	2.1862	
		10.30 P. M.	110	3.546	3.9006	
		7 A. M.	365	4.807	17.5455	
		7.45 P. M.	65	6.25	4.0625	39.005
	Jan. 31 Feb. 1	3 P. M.	265	6.62	17.543	
		12 P. M.	275	6.097	16.7667	
		7.30 P. M.	445	5.882	26.1749	
		2 A. M.	220	5.154	11.3388	71.8234
	Feb. 1–2	10.30 P. M.	575	5.435	31.2512	
		7.30 A. M	455	5.55	25.2833	
		2 P. M.	270	5.102	13.7754	70.3099
	Feb. 2–3[4]	7 A. M.	315	4.198	12.9097	
		7.30 P. M.	190	4.587	8.325	
		2 P. M.	40	2.678	1.1712	22.4059
	Feb. 3–4	10.30 P. M.	135	2.283	3.082	
		7.30 P. M.	445	3.937	17.5196	
		1.30 P. M.	465	5.208	24.172	45.7636
Dog V (*b*)[5]	March 7–8[6]	10.30 P. M.	240	4.902	11.7648	
		7 A. M.	190	4.762	9.0478	
		2 P. M.	200	4.95	9.9	30.712
	March 8–9	11 P. M.	120	3.086	3.7032	
		7 A. M.	320	3.968	12.6976	
		12 M.	80	3.267	2.6136	19.0368
	March 9–10	7 A. M.	160	4.902	7.843	
		9 A. M.	120	5.618	6.7416	
		2 P. M	200	5.25	10.5	25.084
Dog X	May 20–21[8]	5 P. M.	110	3.25	3.575	
		8 A. M.	310	3.52	7.392	
		10 A. M.	90	3.106	2.7954	13.76
	May 21–22	2 P. M.	210	1.004	2.108	
		7.30 P. M.	230	1.348	3.1	
		7 A. M.	330	1.773	5.85	11.058
		10.30 A. M.	95	2.825	2.683	

[1] Complete pancreatectomy Dec. 30, 1910. [2] Injection made Jan. 25, 1911, 2.30 P. M., normal defibrinated blood. [3] Injection Jan. 30, 3 P. M., normal defibrinated blood. [4] Injection Feb. 2, 3 P. M., normal defibrinated blood. [5] Pancreatectomy Feb. 28. [6] Injection March 7, 3 P. M., 150 c.c. normal defibrinated blood. [7] Pancreatectomy May 17. [8] Injection May 21, 10.30 A. M., 150 c.c. normal defibrinated blood.

TABLE II. — *continued.*

	Date.	Time.	Amt. Urine.	Sugar.	Sugar.	Totals.
				per cent.	gm.	
	May 22–23	1 P. M.	85	2.64	2.244	
		5 P. M.	110	2.25	2.475	
		10 A. M.	215	3.50	7.525	14.927
Dog XI (*a*)[1]	June 18–19	10.30 P. M.	130	5.16	6.708	
		8 A. M.	150	8.41	12.615	19.323
	June 19–20 [2]	6 P. M.	180	1.31	2.358	
		10 A. M	120	5.434	65.208	
		8 A. M.	161	5.492	8.787	17.6658
	June 20–21	3.40 P. M.	105	5.747	6.035	
		10.30 P. M.	180	5.434	9.7812	
		8 A. M.	95	5.747	5.457	21.2732
Dog V (*a*)[3]	March 1 [4]	1.30 P. M.	215	4.761	10.2316	
		....	....	....	....	10.2316
	March 1–2	4.30 P. M.	80	0.778	0.6224	
		11 P. M.	70	0.695	0.4865	
		9.30 A. M.	180	3.766	6.778	
		12.30 P. M.	140	5.344	7.4816	15.3685
	March 2–3	3.30 P. M.	165	4.95	8.1675	
		11 P. M.	130	4.672	6.0736	
		7 A. M.	330	4.504	15.764	31.005
Dog XI (*b*)	June 21 [5]	8 A. M.	160	5.492	8.787	
		3.40 P. M.	105	5.747	6.035	
		10.30 P. M.	180	5.434	9.7812	24.6032
	June 22	8 A. M.	95	5.747	5.4578	
		5 P. M.	170	5.263	8.947	
		1.30 P. M.	180	5.371	10.667	25.0726
Dog XII[6]	June 25 [7]	10.30 P. M.	120	4.587	5.5044	
		8 A. M.	200	4.902	9.804	
		2 P. M.	140	4.807	6.7298	22.038
	26	8 P. M.	200	5.435	10.87	
		7 A. M.	120	5.814	6.9768	
		2 P. M.	105	5.747	6.034	23.88
Dog V (*c*)	March 12–13 [8]	7 P. M.	100	4.201	4.201	
		7 A. M.	120	4.673	5.5876	
		7.30 A. M.	130	4.673	6.0749	15.8376
	March 13–14	2.30 P. M.	110	4.807	5.2877	
		10 A. M.	215	4.717	10.1415	15.429
Dog VIII[9]	May 16 [10]	8 A. M.	130	6.024	7.8312	
		10.30 A. M.	170	7.042	11.9714	19.8026
	May 17	4.30 P. M.	105	7.462	7.835	
		11.30 P. M.	25	6.944	1.746	
		7 A. M.	85	6.85	5.9225	15.5035
Dog XII	June 26–27 [11]	8 P. M.	200	5.435	10.87	
		7 A. M.	120	5.814	6.9768	
		2 P. M.	105	5.747	6.034	23.88
	June 27–28	5 P. M.	120	5.618	6.7416	
		7 A. M.	210	5.952	12.499	
		11 A. M.	110	5.882	6.4702	25.7208

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[1] Pancreatectomy June 18, 12 M. [2] Injection June 19, 9 A. M., 150 c.c. normal defibrinated blood. [3] Pancreatectomy Feb. 28, 5 P. M. [4] Injection March 1, 9 A. M., 150 c.c. normal defibrinated blood. [5] Injection June 21, 8 A. M., 100 c.c. NaCl solution 0.8 per cent. [6] Pancreatectomized June 23. [7] Injection June 25, 9 A. M., 150 c.c. blood from diabetic dog. [8] March 13, 10 A. M., injected 150 c.c. blood from diabetic dog. [9] Pancreatectomy May 15, 5 P. M. [10] Injection May 16, 8.30 A. M., 150 c.c. stale blood. [11] Injection June 27, 10 A. M., 150 c.c. stale blood.

with those from which the fall of sugar was obtained, and since there is no change in the sugar percentage it can be taken as proven that the change noted in the experiments above was not brought about by a mere matter of diluting the sugar in the blood of the diabetic animal. (*b*) Since these injections gave no fall in sugar content it seems that the internal secretion is a relatively unstable body and destroyed on standing even for a few hours, hence injections to be successful must be done immediately after the blood is drawn.

A second objection that may be raised is that the change of temperature — the fever that may follow the injections — as has been noticed in the injections of pancreatic extracts, might cause the fall of sugar noticed in these experiments. Tests taken before, during and after the time of the injections, show that the blood injections if done under sterile conditions do not cause any rise in temperature; hence this cannot be a factor. It was noticed, however, that there was a rise of a few degrees after the salt solution injections, but in this case there was not a fall in the sugar percentage.

Nitrogen determinations were not made in all the experiments, but in those in which they were the ratio of D and N was practically that found by Lusk in his experiments, and this proportion, which he considers the only true criterion of the absolute diabetic condition, runs a course comparable to the course of the sugar given above.

Summary of Results.

1. Intravenous injections of fresh defibrinated blood from a normal dog into a pancreatectomized dog lower the percentage of sugar in the urine and the D : N ratio for a period of less than twenty-four hours. Control experiments made with salt solution, stale blood, and blood drawn from pancreatectomized animals show that this action is due to the internal secretion of the pancreas present in the blood.

2. The defibrinated and sterile blood loses this action in the course of a few hours. The internal secretion of the normal pancreas, then, appears to be a relatively unstable body, a fact which may make its extraction from the gland difficult, and may account for some of the failures to secure active extracts from the pancreas.

STUDIES IN EXPERIMENTAL GLYCOSURIA. — VII. THE AMOUNT OF GLYCOGENASE IN THE LIVER AND IN THE BLOOD ISSUING FROM IT, AS AFECTED BY STIMULATION OF THE GREAT SPLANCHNIC NERVE.

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THE variable amounts of sugar which are discharged by the liver into the blood of the inferior vena cava depend on variations in the activity of the intracellular diastatic ferment, glycogenase. A marked hyperactivity of this ferment, resulting in a rapid rise in the percentage of sugar in the blood, can be produced by stimulation of the nervous system in various ways, *e. g.*, piqûre, stimulation of the splanchnic nerve, etc. The hyperglycæmia which occurs in the different forms of experimental diabetes (pancreatic, adrenalin, etc.) and in diabetes mellitus is also due, in the earlier stages at least, to a similar cause, although later in these cases the process, which is responsible for the excessive sugar production, includes something more than exaggerated glycogenolysis.

These variations in the activity of glycogenase may depend on: (1) a variation in the amount of the active ferment or (2) a variation in the conditions under which a constant amount of ferment acts. It is generally assumed that it is by the former of these possible mechanisms that the variations in sugar production in the liver occur, and in support of such a view, Bang and his pupils have published extensive researches which would at first sight seem to support the hypothesis. We shall discuss Bang's observations later, but meanwhile it will be well to point out that the willingness of most writers to accept such a view without much questioning is due to its undoubted application in the case of the digestive glands. As has been shown by Pawlow, the amount of active ferment produced by these varies more or less with the amount of food to be digested. To draw an analogy between the action of the digestive glands and the glycogenic function of the liver is, however, quite unwarranted, for the former concerns the production of extra-cellular enzymes over which, after their secretion, the cell which produces them can have no control, whereas the latter concerns the activity of a ferment acting where it is produced and susceptible to all sorts of controlling influences exercised by the cell in which it acts. In the one case the secretion is external, in the other we are dealing with a ductless gland.

When the active secretion of a ductless gland is carried away by the blood, it is probable that its production may be under the control of the nervous system, *e. g.*, the secretion of adrenalin;[1] but when the active secretion unfolds its action within the body of the cell which produces it, as is believed to be the case with glycogenase, other methods of regulation than a variation in the amount of ferment must be considered as responsible for the variable activity. In such cases we should expect the amount of active ferment extractable from the gland to undergo no change even although the gland is hyperactive. It is not the amount of intracellular ferment which varies as the result of nerve stimulation, but the conditions under which a constant amount of ferment acts. Such changes in environment can be produced in a multitude of ways, *e. g.*, changes in the H′-ion concentration in the cell, changes in the nature of the kations present, the presence or absence of co-ferments and activators and in the case of certain ferment processes at least, changes in the physical condition of the substrat (*i. e.*, in the manner in which it is contained in the protoplasm of the cell).

These preliminary remarks will indicate the nature of our problem in the present research. Briefly stated, it is this: is the amount of glycogenase extractable from the liver cells, when these are producing excessive amounts of sugar from glycogen, any greater than that extractable from the normal liver? Since it is known that certain of the ductless glands discharge their secretion into the blood, we have also in the present research examined the blood issuing from the liver to see whether an increased amount of glycogenase might be present in it during excessive production of sugar by the liver.

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[1] *Cf.* Swale, Vincent: Ergebnisse der Physiologie; Jahrg. IX, 1910; Asher: Zentralblatt für Physiologie, 1911, No. 20, p. 927.

As already stated, Bang, Ljungdahl and Bohm[2] have published researches bearing on the same question. Rabbits, usually fed with sugar, were quickly killed and the liver of each washed free of blood with isotonic saline solution at body temperature. The blood-free liver was then minced and portions incubated in the presence of toluol for four hours at body temperature, after which the amount of glycogen was determined. Another portion of each liver was heated so as to destroy its ferments; it served as a control of the amounts of glycogen originally present. When the liver did not contain much glycogen, this substance was sometimes added to the minced liver so as to keep the amounts constant. The difference between the amounts of glycogen found in the heated and the incubated portions of liver was taken as an index of the activity of the glycogenase present in the liver at the time of death. This difference was calculated as a percentage of the amount of glycogen at the start of incubation. Such experiments were carried out with normal rabbits and with rabbits that had been treated, just before killing, in some way known to produce glycosuria. A series of remarkable results were obtained, of which the following may be noted in the present connection. The normal percentage disappearance of glycogen in six hours was found to vary from 6.6 for rabbits in winter to 9.4 for rabbits in summer. Starvation was found to increase these figures to an average of 13. Asphyxia raised it to 13.8, and hemorrhage from the carotid with simultaneous transfusion of saline through the jugular vein yielded values ranging from 12.1 to 35.3. Great increases were produced by stunning the rabbit; piqûre caused a sudden great increase followed by a fall which was again followed by a rise. Without devoting much time to a discussion of those results, there are several points in connection with them which require some attention. In the first place the individual figures from which the averages are compiled, are extremely irregular; for normal rabbits, for example, they vary from 2.3 to 9.3 per cent (winter rabbits) and 7.1 to 11.0 (summer rabbits). Indeed in Tables I, III, and IV, the absolute amounts of glycogen which disappeared during incubation are distinctly more constant than the percentage amounts, and when we consider those values in the case of the experiments in which increased ferment content is supposed to be demonstrated, no constant change is noticeable. The averages given for absolute amounts of glycogen which disappeared from normal liver are 0.67, 0.96, and 0.87; for the starvation experiments, 0.439; for transfusion with various saline solutions, 0.56, 1.27, 0.78, 1.01; for hemorrhage, 1.15, and for asphyxia, 1.23. The initial amounts of glycogen in the above cases were respectively 12.2, 10.5, 13.0; 0.845; 4.6, 8.2, 5.88, 6.40; 8.20, and 6.80. The variations which the percentage values reveal were therefore more dependent on the original amounts of glycogen present in the incubation mixtures than on the amounts which actually underwent change.[3]

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[2] Bang, I. (Ljungdahl and Bohm): Beiträge zur chemischen Physiologie und Pathologie, 1907, ix, p. 408; 1907, p. i; 1907, x, p. 312.

Quite apart from these errors in method of computation, however, the principle adopted for the estimation of the amount of ferment is wrong, for such changes as were observed might be due to several causes. Thus, variations in the reaction of the incubation mixture would have a marked effect on the rate of glycogenolysis (for it is improbable that the amount of the acids produced by autolysis in different livers is the same).[4] The presence in the incubation mixtures of variable amounts of substances capable of influencing the activity of glycogenase, such as lecithin[5] and bile salts,[6] as well as of antiferments originally present in the blood and lymph, but not always removed by the washing process to the same degree, might account for the differences observed. The same criticism would apply to the removal of the blood and lymph ferments.[7] The greater glycogenolytic power of livers removed from rabbits perfused through the jugular vein with saline solution as compared with "normal" livers might be explained by unequal washing out of blood from the liver in the two cases.

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[3] In this connection it is of importance to bear in mind that when there is an excess of substrat in comparison with the amount of enzyme present in the reaction mixture of starch and diastase, the amounts of starch hydrolyzed in equal periods of time are equal. In such a case it is obviously wrong to use the percentage amount hydrolyzed as a criterion of the strength of ferment.

BROWN, H. T., and GLENDINNING, T. A.: Journal of the Chemical Society, 1902, lxxxi, p. 388.

[4] *Cf.* MACLEOD, J. J. R.: This journal, 1909, xxiii, p. 278.

[5] CENTANINI: Biochemische Zeitschrift, 1910, xxix, p. 389; LAPIDUS: Biochemische Zeitschrift, 1910, xxx, p. 39.

[6] WOHLGEMUTH, J.: Biochemische Zeitschrift, 1909, xxi, p. 447.

[7] BANG seems to recognize these possible influences, for he discusses them in connection with alcohol precipitation of the ferment. By his method, however, he does not avoid them.

In most of Bang's experiments there is no evidence that when abnormally large amounts of ferment were found present any larger than normal amounts of sugar were being produced by the liver.

The comparisons were made between the livers of normal and experimentally treated animals. This cannot of course yield reliable results unless very large numbers of investigations are made. Some of Bang's conclusions are based on the results obtained from one or two experiments (*cf.* Tables 7, 8, 9, 10, and 11 of Bang's paper).

In the present research the technique adopted has varied somewhat in different groups of experiments. It is of course impossible to obtain pure preparations of glycogenase, the precipitates with alcohol, as used by Pick, Mendel, etc., being quite uncertain as regards their glycogenolytic activity. Thus we have found that alcohol precipitates of the same liver may show quite variable glycogenolytic strengths according to the length of time during which the precipitate has stood under the alcohol. On the other hand, as already explained, it is necessary to isolate the glycogenase as far as possible from the other constituents of the liver cell which could influence its activity.

We have attempted to fulfil these conditions in the present research, firstly, by using liver preparations which were brought as little as possible in contact with reagents, such as alcohol, which can affect the activities of glycogenase; secondly, by allowing the ferment preparations to act in the presence of a relatively large volume of pure glycogen solution, which was the same in amount for the different ferment preparations of a given experiment; thirdly, by maintaining the reaction feebly acid by the use of a phosphate mixture. In one set of experiments saline or Buchner extracts of liver were employed; in another set, Wiechowski preparations.[8]

In order to eliminate errors involved by comparing preparations from the livers of different animals, we have chosen for our work sufficiently large dogs to permit of the removal at the beginning of each experiment of a portion of liver from which a determination of the normal ferment-strength could be made. The glycogenolytic activity of this normal was then compared with that of an exactly similar preparation from another portion of liver removed during stimulation of the great splanchnic nerve. We have employed this method for exciting increased sugar production in the liver because it is the most direct and uninvolved that we know of.

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[8] WIECHOWSKI: ABDERHALDEN'S Handbuch der Biochemischen Arbeitsmethoden, 1909, iii, 1, p. 282.

In all cases we have collected blood from the inferior vena cava opposite the entrance of the hepatic veins before, and during, the stimulation of the nerve and determined the percentage of reducing substance in it, in order to be certain that increased glycogenolysis was really induced by the splanchnic stimulation.

The details of technique are as follows:

A diet of bread and meat, sometimes with cane sugar added, was fed to the dogs on the day previous to the experiment. After etherizing, tracheal and carotid cannulæ were inserted, the latter being connected, by means of tubing filled with two per cent sodium citrate solution, with a mercury manometer. The splanchnic nerve on the left side was then exposed and laid, without cutting it, on shielded electrodes. A ligature was placed underneath the inferior vena cava just below the renal veins, and a glass cannula about 8–10 cm. long and 6 mm. external diameter inserted in the central end of the left renal vein. The open end of this cannula lay in the inferior vena cava about opposite the entrance of the hepatic veins. It was connected by rubber tubing with a T-piece and served for the collection of blood from the vena cava. In collecting the blood samples the following procedure was adopted: a powerful syringe made of hard rubber and of a capacity of 50 c.c. was connected with one limb of the T-piece, the remaining free limb being closed by rubber tubing and a clip. A few cubic centimetres of blood were sucked into the syringe, the tubing between the cannula and the T-piece then clamped, the free limb of the T-piece opened, and the aspirated blood ejected through it. This process was repeated three times so as to be certain that there should be no contamination of the blood samples with fluid present in the tubing or syringe. The ligature under the vena cava was then pulled up so as to shut off the blood from the lower portion of the body and the piston of the syringe slowly withdrawn. This process occupied about thirty seconds. The ligature under the vena cava was immediately loosened, the tubing between cannula and T-piece clamped, the syringe removed and the blood discharged into Reid's reagent or 2 per cent potassium oxalate solution. A few cubic centimetres of a 2 per cent solution of sodium citrate was then sucked into the syringe, this connected again with the T-piece, and most of the solution discharged through the free limb, after which a little of the solution was injected into the vein through the cannula. By this method the cannula and tubing were freed of blood and filled with the anti-coagulant fluid. The removal of the subsequent samples of blood was in this way easily accomplished without any trouble with clots. The small amount of sodium citrate solution which was injected in the circulation did not, as control experiments showed, cause any increase in the percentage reducing power of the blood.

Immediately after the removal of the blood sample, a lobe of liver was removed, a flat ligature (shoe lace) being tied around its base. The piece of liver was either washed free of blood by means of isotonic saline injected through the veins exposed in the cut, or was cut in thin slices which were washed in several changes of isotonic saline and pressed firmly several times between folds of absorbent filter paper until these no longer were stained by blood. In a given experiment of course either the one or the other of these methods for the removal of blood was adopted. All traces of blood and lymph were probably not removed by the filter paper method, and even by that of perfusion it is uncertain whether this can be equally effected in all cases. The one method is probably as reliable as the other, however, and the subsequently recorded results will indicate of what value the adoption of both proved to be in the present research. The blood-free liver was then quickly weighed, and in most of the experiments passed through a fine disintegrating apparatus and immediately spread out in a thin layer on glass plates (2.5 dm. × 4 dm.), which were then placed in a strong current of air in a suitably made air-tight box connected with a powerful air pump. After drying (which occupied from 2 to 4 hours) the scales of dried liver tissue were either immediately used for preparing the ferment solution or placed in a cartridge of absorbent paper, such as are used for fat extractions, and extracted with cold toluol in Wiechowski's apparatus for several days.[9] The residue was finally ground thoroughly in a mortar and, for the incubation experiment, 1 gm. macerated with 10 c.c. distilled water until an emulsion was obtained.

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[9] WIECHOWSKI: *Loc. cit.*

The advantages of Wiechowski's method for work of the type described in this paper are: (1) that it requires only a small piece of tissue; (2) that the final preparations can be kept indefinitely; (3) that no reagent capable of injuring the diastase is used; (4) that lipoids are removed, thus diminishing the tendency to change of reaction during subsequent incubation, on account of the action of lipase.

In others of the experiments a larger lobe of liver was removed, and a Buchner or a saline extract prepared from it as described in a previous paper.[10]

The incubation experiments were also conducted in the same way as described in the paper referred to with the following modifications.

1. The incubation mixtures were all mixed with a phosphate solution made by adding to a m/3 solution of H_3PO_4 15/16ths of the amount of N/10 NaOH necessary to give the same tint with phenolphthalein as is given by a solution of Na_2HPO_4 and phenolphthalein of similar concentration. This contains practically the same concentration of H'-ions as blood. The amount of this solution added was in each case sufficient to give the same percentage of H_3PO_4 in the incubation mixture as exists in blood (0.01 per cent). During incubation the solutions remained practically isohydric, as was ascertained by making comparative titrations of equal amounts of certain of the incubation mixtures with rosolic acid before and after incubation. Before adopting these quantities of phosphate mixture we investigated the influence on the rate of glycogenolysis of varying amounts of the mixture. The following results were obtained: Quantities of 25 c.c. each of a glycogen solution were mixed with an amount of Wiechowski liver preparation equalling 1 gm. moist liver, varying quantities of phosphate mixture added and incubated; 2 cm. were removed from each flask before and after incubation, diluted and mixed with equal amounts of rosolic acid. The tint was, if anything, somewhat darker in the incubated specimens. There was certainly no increase in acidity in any of the samples. Twenty cubic centimetres of each specimen were then used to determine the rate of glycogenolysis with the following results:

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[10] Macleod, J. J. R., and Pearce, R. G.: This journal, 1910, xxv, p. 255.

1. Contained 0.2 c.c. phosphate solution and showed 31.7 per cent glycogen disappeared.
2. Contained 0.3 c.c. phosphate solution and showed 33.1 per cent glycogen disappeared.
3. Contained 0.4 c.c. phosphate solution and showed 29.4 per cent glycogen disappeared.
4. Contained 0.5 c.c. phosphate solution and showed 28.8 per cent glycogen disappeared.
5. Contained 0.7 c.c. phosphate solution and showed 29.4 per cent glycogen disappeared.
6. Contained 0.9 c.c. phosphate solution and showed 29.3 per cent glycogen disappeared.
7. Contained 1.2 c.c. phosphate solution and showed 29.8 per cent glycogen disappeared.

The rate of glycogenolysis was practically the same throughout. In our experiments we have added 0.22 c.c. phosphate mixture for every 10 c.c. of incubation fluid. This corresponds with No. 3 in the above table.[11] We have found that a large excess of phosphate sometimes accelerates glycogenolysis independently of the reaction; hence the reason for using such small amounts in these experiments.[12]

2. In a great majority of the experiments the contents of the flasks were kept in constant agitation during incubation by means of a mechanical shaking device placed in the incubator. We have adopted this precaution because of the sediment which otherwise not infrequently collects in the incubation mixtures. These sediments contain glycogen. The same precaution is also urged by Starkenstein.[13]

3. The glycogen estimations were made in the same way as in our previous paper except that Bang's titration method was employed instead of the gravimetric, for the estimation of the dextrose. It is impossible to use, for this research, any approximate method for the determination of diastatic activity. Of such methods that of Wohlgemuth is usually employed, but, although very useful for many purposes, it is not accurate enough to demonstrate the slight differences in ferment strength which might exist in our experiments.

In calculating the results, we have assumed that the amount of glycogen in each of the solutions in a given experiment was the same. It is true that during the time which intervened between the removal of different portions of liver — usually only a few minutes (see tables) — some glycogen must have disappeared, but when we consider that in practically all of the experiments an amount of extract was used amounting only to 1.0 gm. moist liver, it is evident that any error thus incurred will be entirely negligible.

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[11] We are greatly indebted to Dr. L. J. HENDERSON for the valuable advice which he gave us concerning the use of phosphate mixtures.

[12] Unpublished experiments.

[13] STARKENSTEIN, E.: Biochemische Zeitschrift, 1910, xxiv, p. 191.

To express the amount of ferment in the different preparations we have given the percentage amounts of glycogen disappearing in equal periods of time. Since there is a division of opinion among authors[14] as to whether the concentration of diastatic ferment varies directly with the amount of substrat decomposed or according to the law of Schutz and Borrisow, it is of course impossible to calculate the relative amounts of ferment in two preparations showing varying activities. On the other hand, if equal amounts of ferment solutions produce the *same* percentile glycogenolysis, it can be concluded that the same amount of ferment is present in the two.

One serious difficulty in all work of this nature is the measurement of the amount of ferment preparation employed in the different flasks. In practically all the experiments we have weighed 1 gm. of the dry liver powder and ground it in a mortar with 10 c.c. water. Of the resulting emulsion, 2 c.c. were added to the glycogen solutions. As a criterion of the actual amount of liver substance added to each flask, we have used the percentage of nitrogen in the ferment preparations, and we have calculated the percentile glycogenolysis in terms of the same amount of nitrogen.[15] In doing this we are aware that if the amount of glycogen decomposed varies as the square root of the ferment present — which as above indicated is uncertain — we are incurring an error at least in those cases in which there is an appreciable difference in the amount of nitrogen in the different preparations. In the majority of our experiments, however, the ferment preparations of a given experiment contained practically the same amount of nitrogen, so that there can be no very great error, due to this method of calculation. The only exceptions to this statement exist in the case of Buchner extracts (see p. 447); although prepared in the same way, these often varied considerably in nitrogen content.

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[14] TAYLOR: On fermentation, Berkeley, 1907; HENRI, *cf.* HANS EULER, Ergebnisse der Physiologie, 1910, ix$^{\text{er}}$ Jahrgang, p. 270; BROWN and GLENDINNING: Journal of the Chemical Society, 1902, lxxxi, p. 381; KLEMPIN: Biochemische Zeitschrift, 1908, x, p. 206.

[15] TAYLOR, A. E.: Journal of biological chemistry, 1908, v, p. 315.

We shall first of all consider the results relating to the ferment strength of Wiechowski preparations of portions of liver removed before, during, and subsequent to stimulation of the great splanchnic nerve. Along with these we shall consider the behavior of the serum from the blood of the vena cava under the same conditions.

In the *fourth* column of Table I are given the percentage amounts of reducing substance in the blood of the vena cava opposite the liver.[16] Two facts of importance are established by the results: 1. When the animal was quietly under ether, the reducing power of the blood either remained practically constant (as in the first part of Experiments XXIV and XXV) or decreased slightly in amount (as in Nos. XII and XIV). In a total of seventeen experiments (including those in the above table) in which the reducing power of the blood of the inferior vena cava was estimated at intervals of about thirty minutes apart and during which there was no nerve stimulation, ten (60 per cent) showed a slight decrease, four remained constant (23 per cent) and three showed a slight increase (17 per cent). 2. When the splanchnic nerve was stimulated, a distinct increase in reducing power occurred in six of the seven experiments in which this was done. In Experiment XVIII no increase in reducing power occurred during the stimulation, and it is noted in this case that the blood pressure was very slightly affected. Although the electrodes were properly applied to the nerve, which was ascertained by autopsy, there cannot have been adequate stimulation of the nerve.

The *fifth* column shows the percentage of glycogen which disappeared when 1 c.c. of serum was incubated with 10 c.c. of 2 per cent glycogen in isotonic sodium chloride solution for periods of time varying from two to three hours. It is evident that the glycogenolytic power of the blood was not affected by the nerve stimulation. The only exception is in the case of Experiment XVIII, where a distinct increase in glycogenolytic power was noted in blood collected thirty minutes after the removal of the stimulus. The slight variations, amounting to about 4 per cent, observed for the

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[16] Waymouth Reid method.

TABLE I.

Percentile Glycogenolysis in Blood Serum and Liver Preparations as affected by Stimulation of the great Splanchnic Nerve.

No. of expt.	Time.	Experimental condition.	Per cent sugar in blood.	Glycogenol'sis. Blood.	Glycogenolysis. Liver.	Remarks.
XII	2.45 P. M.	Ether anæsthesia	...	...	28.8	5 hrs. incubation.
	2.50 P. M.		0.166	...	...	
	3.00 P. M.		0.142	...	...	
	3.25 P. M.		0.140	...	...	
	3.55 P. M.		0.146	...	...	
	4.00 P. M.		...	...	29.2	
XIV	2.30 P. M.	Ether anæsthesia	0.165	71.0	...	5 hrs. incubation.
	3.50 P. M.		0.124	75.0	...	
XVI	10.50 A. M.	Ether anæsthesia	0.136	...	...	* Not in terms of nitrogen. 8 hrs. incubation.
	10.55 A. M.		...	...	77.7 *	
	11.03 A. M.	Stim. of gr't splanchnic nerve	...	...	...	
	11.10 A. M.		0.176	...	...	
	11.14 A. M.		...	...	70.0	
	11.26 A. M.	....	0.184	...	...	
	11.30 A. M.	....	...	...	77.0	
XVIII	2.40 P. M.	Ether an'sia	0.124	72.8	62.7	Fed meat and bread. Only small rise in blood pressure on stimulating.
	2.45 P. M.	Stim. of gr't splanchnic nerve	...	...	...	
	2.50 P. M.		...	73.7	64.2	
	2.52 P. M.		0.110	...	...	
	3.25 P. M.	....	0.144	82.6	...	
	3.28 P. M.	....	....	...	64.2	
XIX	11.25 A. M.	Ether anæsthesia	0.130	67.4	...	Fed bread and meat. Marked rise in blood pressure.
	11.27 A. M.		...	...	79.5	
	11.30 A. M.	Stim. of gr't splanchnic nerve	...	...	...	
	11.37 A. M.		...	...	72.1	
	11.40 A. M.		0.176	66.1	...	
	12.09 A. M.	....	0.148	68.5	...	
	12.11 A. M.	....	...	...	79.8	
XX	10.28 A. M.	Ether anæsthesia	0.199	52.1	...	Given sugar on preceding day.
	10.30 A. M.		...	...	24.1	
	10.35 A. M.	Stim. of gr't splanchnic nerve	...	...	...	
	10.40 A.M.		0.265	50.7	...	
	10.45 A. M.		...	...	...	
	11.15 A. M.	....	0.415	49.6	...	
	11.17 A. M.	....	...	...	24.1	
XXI	10.40 A. M.	Ether anæsthesia	...	...	...	Given sugar on preceding day.
	11.05 A. M.		0.151	40.8	41.4	
	11.10 A. M.	Stim. of gr't splanchnic nerve	...	...	...	
	11.17 A. M.		0.195	35.6	...	
	11.21 A. M.		...	...	52.0	
	11.45 A. M.	....	0.168	40.3	...	
XXIV	10.19 A. M.	....	0.131	...	...	Given sugar on preceding day.
	10.21 A. M.	....	...	...	48.1 44.9	
	10.40 A. M.	....	0.121	...	... ...	
	10.42 A. M.	....	...	...	46.1 ...	
	10.43 A. M.	Stim. of gr't splanchnic nerve	...	...	... ...	
	10.50 A. M.		0.154	...	... ...	
	10.52 A. M.		...	...	53.3 51.1	
XXV	10.45 A. M.	....	...	...	... ...	Given sugar on preceding day.
	11.10 A. M.	....	0.194	...	76.7 70.0	
	11.40 A. M.	....	0.213	...	.... ..	
	11.44 A. M.	Stim. of gr't splanchnic nerve	...	...	... ...	
	11.49 A. M.		0.280	...	... ...	
	11.51 A. M.		...	...	73.1 71.4	

others, undoubtedly indicate the experimental error involved in the method.

In the *sixth* column is given the percentile glycogenolysis induced by incubating 2 c.c. of a watery emulsion of toluol-extracted liver powder with 10 c.c. of 2 per cent glycogen in the presence of phosphate mixture and an isotonic quantity of sodium chloride. The incubation lasted from five to eight hours. In the absence of any nerve stimulation, a variation amounting in one case to about 5 per cent (Nos. XII and XXIV) was observed between different preparations of the same liver. In the seven experiments in which the splanchnic nerve was stimulated, no increase in ferment strength was observed in five (Nos. XVI, XVIII, XIX, XX, and XXV); but in the remaining two (XXI and XXIV) a distinct increase over the normal was evident in the ferment preparations from stimulated liver.

Before concluding that the greater glycogenolysis in these two experiments is due to an actual increase in the intracellular glycogenase of the liver cell, several possibilities have to be borne in mind. The first of these is an unusually large experimental error in the incubation experiments. A repetition of the incubation experiments with other portions of the liver powders, however, yielded practically the same results, as the duplicate figures given in the table show. A third repetition of the incubation experiments with liver preparations of the same experiments that had merely been air-dried, but not extracted with toluol, also yielded corresponding results.

A second source of error which might account for the increase in these two experiments lies in the fact that it is improbable that the blood can be washed out from all the portions of liver to an equal degree (see p. 439). Since blood contains a very active glycogenase — much more so than liver tissue — a small trace of it left in the liver would have a marked effect on the glycogenolytic strength of the extract. We have recognized this source of error throughout our work and have tried to guard against it by washing the various portions of liver to an equal degree. We could not wash the specimens through their blood vessels because they were too small.

A third condition which must be thought of is unequal post-mortem change in the different portions of liver, for it is known that a distinct increase in glycogenolytic power occurs in dead liver.

The indecisive nature of these results made it necessary for us to repeat the experiments by the use of ferment preparations prepared in different ways. The several unknown possible sources of fallacy involved in such work as the present can be discounted only by frequent repetition by the use of different methods, and since we consider the question — is there, or is there not, an increase in glycogenase in the liver during splanchnic stimulation? — a fundamental one for our farther progress, we have devoted more extended research to its solution.

In the first place it might be possible that the treatment with cold toluol had so altered the ferment as to make it appear equal in amount in the majority of the experiments. In other words, the nerve stimulation might really have caused an increase in ferment which the treatment with toluol had rendered undemonstrable.

On repetition of certain of the above experiments with unextracted liver powder, the following results were obtained: In Experiment XX there was 25.9 per cent glycogenolysis before stimulation and 26.3 during it. In Experiment XXI there was 46.4 per cent glycogenolysis before stimulation and 56.3 in a portion of liver removed twenty-three minutes after it was discontinued. In Experiment XXIV the duration of incubation in the case of the non-extracted preparations was shorter than for those given in the table, and gave 30.7 per cent glycogenolysis for the first and 38 per cent for the second portion of liver; in the portion removed during splanchnic stimulation there was 43.2 per cent glycogenolysis. In Experiment XXV there were also three portions of liver, two of which were removed before and one during stimulation. The first two gave 73.8 and 74.3 per cent glycogenolysis and the third 75.5 per cent. It is seen that the results were practically identical with those recorded in Table I. They show us further that the lipoid substances in the liver do not influence the activities of glycogenase.

In another series of experiments the following modifications in the above-described procedure were introduced: 1. Larger portions of liver were removed so that, by introducing a cannula in the blood vessels, they could be washed thoroughly free of blood.

2. The extracts were prepared immediately after removal of the portions of liver, either by thorough maceration with fine sand and isotonic saline in a mortar or by Buchner's process.

3. Instead of taking one specimen of each preparation and incubating for a given time, three specimens of each were taken and incubated for variable times. This was done to see whether any differences in ferment strength might not reveal themselves during the earlier stages of the glycogenolytic process, although they had become masked after longer action of the ferment, when this was approaching completion. The results are tabulated in Table II.

TABLE II.

THE COMPARATIVE GLYCOGENOLYTIC POWER OF EXTRACTS OF LIVER PREPARED IN VARIOUS WAYS, BEFORE AND DURING STIMULATION OF THE SPLANCHNIC NERVE.

No. of expt.	Time.	Experimental condition.	Mode of preparation of extract.	Percentile glycogenolysis for equal amounts of nitrogen.
XXXIV	10.30 A. M.	Blood and liver removed[1]	Air-dried liver powders. Liver not perfused	67.0
	11.03 A. M.	Liver removed		65.4
	11.05 A. M.	Stim. of great splanchnic nerve	do.	65.7
	11.26 A. M.	Liver removed		
XXXV	10.00 A. M.	....	Liver ground thoroughly in mortar with sand and water. Liver not perfused	
	10.40 A. M.	Blood removed[2]		
	11.13 A. M.	Liver removed		40.9
	11.18 A. M.	Stim. of great splanchnic nerve		
	11.25 A. M.	Liver removed		40.1
XXXIX	10.35 A. M.	Liver removed	Buchner extract of liver. Liver perfused through vessels with isotomic saline	33.6[3]
	10.45 A. M.	Stim. of great splanchnic nerve		
	10.55 A. M.	Liver removed		28.3
XL	10.30 A. M.	Liver removed	Buchner extract of perfused liver	90 min. 13.5 180 min. 27.0 420 min. 48.5
	10.32 A. M.	Stim. of great splanchnic nerve	do.	90 min. 13.4 180 min. 30.0 420 min. 42.5[4]
	10.45 A. M.	....		

[1] Blood serum caused same amount of glycogenolysis before and during stimulation.

[2] Blood serum caused same amount of glycogenolysis before and during stimulation. Fed bread and meat.

[3] Not in same terms of nitrogen, but according to volume of extract used. Fed bread and meat.

[4] Fed bread and meat.

Apart from the fact that the results of these four experiments agree with the majority of those recorded in the previous table, in showing no increase in ferment in liver removed during splanchnic stimulation, they are of further interest because in two of them, *viz.*, Nos. XXXIX and XL, all traces of blood were removed from the pieces of liver, by perfusion through the blood vessels with isotonic saline solution. It has already been suggested that the increases in ferment strength obtained in Experiments XXI and XXIV of Table I in portions of liver removed during splanchnic stimulation might be due to faulty removal of blood by the less thorough method employed in the cases. The results recorded in Table II would seem to confirm this view.

Another possible source of fallacy in these experiments has to be considered, namely, a development of ferment as a result of the death of the liver cells. We have shown in a previous volume of this journal[17] that a moderately rapid disappearance of glycogen sets in about twenty minutes after death and then proceeds in accordance with the laws which govern a monomolecular reaction, suggesting therefore that after this post-mortem glycogenolysis has once become established, the amount of ferment remains constant, the only thing to change being the amount of unhydrolyzed glycogen. We have suggested, and so have Pavy and Bywaters,[18] that this post-mortem acceleration in the glycogenolytic process is due to the development of acids in the liver. Since, however, it is only a surmise, and by no means an established fact, that there is no actual increase in glycogenase in the liver after death, we have thought it well, before concluding this research, to repeat the above experiments with pieces of liver washed blood-free by ice-cold saline, so as to prevent any post-mortem change. In the event that a post-mortem production of glycogenase did occur, it would probably be equal in resting and stimulated liver, for in both cases its derivation would be from the same store of "mother" substance. In such a case it is evident that any increase in active ferment due to splanchnic stimulation would be masked by the post-mortem process.

After etherizing and applying the electrodes to the splanchnic nerve a ligature was tied around the left central lobe near its base and the lobe cut away and immediately cooled in ice-cold isotonic saline solution. A suitable cannula was then inserted in the various cut veins and the portion of liver washed blood-free, after which a Buchner or a saline extract was prepared, using an ice-cold mortar so as to prevent any rise in temperature. The incubation periods were made of variable durations for the reason already explained (see p. 417). Phosphate mixture was used only in certain of the specimens.

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[17] Macleod, J. J. R., and Pearce, R. G.: This journal, 1911, xxvii, p. 341.

[18] Pavy, F. W., and Bywaters, H. W.: Journal of physiology, 1910, xli, p. 168.

TABLE III.

PERCENTILE GLYCOGENOLYSIS OF SALINE AND BUCHNER EXTRACTS OF RESTING AND STIMULATED LIVERS, PREPARED AT LOW TEMPERATURES.

No. of expt.	Experimental condition.	Nature of extract.	Per cent nitrogen.	Percentile glycogenolysis in incubation mixtures.		Remarks.
				min.		
LX	Ether anæsthesia	Buchner	1.10	120	32.1	Dog fed bread and meat. No phosphate added.
				210	49.2	
				300	63.9	
	Stimulation of great splanchnic nerve	....	1.20	120	31.8	
				210	45.2	
				300	59.0	
LXIII	Ether anæsthesia	Saline	1.22	90	18.7	Dog starved two days before exp. No phosphate added.
				180	...	
				300	61.2	
	Stimulation of great splanchnic nerve	....	1.17	90	25.6	
				180	44.2	
				300	61.0	
LXIV	Ether anæsthesia	Saline	....	90	21.8	Dog fed bread and meat. No phosphate added.
				180	42.77	
				300	52.2	
	Stimulation of great splanchnic nerve	....	....	90	20.1	
				180	42.5	
				300	51.7	

The results recorded in Table III confirm those of the preceding tables in showing no increase in ferment as a result of splanchnic stimulation.

The conclusion which is warranted by the results of the experiments recorded in this paper is that increased glycogenolytic activity in the liver is not due to an increase in the amount of glycogenase in the liver cells. By exclusion we are compelled to conclude that it is by an adjustment of the conditions under which a constant amount of glycogenase is acting that variations in glycogenolytic activity are brought about. It is not our intention in this paper to enter into any theoretical discussion of the possible nature of these changes. Several possibilities have already been indicated on page 403. In weighing the possibilities one fact should be borne in mind, namely, the manner in which glycogen is stored within the protoplasm of the liver cell. It is supposed by certain histologists [19] to be combined with a peculiar form of sustentacular or holding material (Trägersubstanz). If this be the case, it can readily be imagined that, while thus deposited in the liver cell, the glycogen is inaccessible to the action of glycogenase, and that the degree to which it can be set free is, either directly or indirectly, under the control of the nervous system. Whenever it is set free, glycogenase can act on it. Such an hypothesis does not of course specify of what nature the holding substance may be; it may be some substance acting as an antidiastase, or it may be some inert substance which by uniting with the glycogen prevents the union with it of the glycogenase. Suggestions of this nature have been made by McGuigan and Brooks,[20] who suppose that in the liver cell glycogen is in combination with protein and must become dissociated from it before it can be hydrolyzed. Certain salts such as those of calcium render this compound more stable and therefore tend to prevent glycosuria; others encourage the destruction and therefore excite glycosuria.

E. Frank and S. Isaac [21] also place great importance on the fixation of glycogen with some constituent of the liver cell. So long as this union is a firm one, the diastatic ferment is incapable of attacking the glycogen: when it becomes weakened — as they believe it to be during a certain stage of phosphorus poisoning — the diastatic ferment converts the glycogen into sugar.

CONCLUSIONS.

Stimulation of the splanchnic nerve in the dog, although it causes a marked increase in the reducing power of the blood of the vena cava opposite the liver, does not cause any increase in the glycogenolytic power of extracts of liver prepared in various ways. The blood issuing from the liver also possesses the same glycogenolytic power before and during stimulation of the nerve.

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[19] ARNOLD: VIRCHOW, Archiv für pathologische Anatomie und Physiologie, 1908, cxciii, p. 174.

[20] MCGUIGAN and BROOKS: This journal, 1907, xviii, p. 256.

[21] FRANK, E., and ISAAC, S.: Archiv für experimentelle Pathologie und Pharmakologie, 1911, lxiv, pp. 274, 292.

It is concluded, that modifications in the glycogenolytic activity of the liver do not depend on changes in the amount of glycogenase, but on changes in the conditions under which a constant amount of this ferment is acting.

METABOLISM OF DEVELOPMENT. — III. QUALITATIVE EFFECTS OF PREGNANCY ON THE PROTEIN METABOLISM OF THE DOG.

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PRACTICAL as well as theoretical interest in the metabolism of pregnancy has grown very rapidly during the past few years. Evidence has been adduced by Massin,[1] Zweifel,[2] Stone,[3] Edgar,[4] Ewing and Wolf,[5] and others, that some of the worst forms of the toxemia of pregnancy, including eclampsia, are due to functional disturbances in those organs which are responsible for the metabolism of the protein substances; and a considerable expectancy has been created that detailed urinary analyses for the protein end-products would enable the obstetrician to detect, and therefore possibly to avert, these maladies.[6]

Unqualified acceptance of these findings as indicative of the degree of abnormality, however, waits upon more perfect knowledge of the metabolism of normal pregnancy. Some of the observers abovementioned, notably Massin and Ewing, the former proceeding from his experience with the Eck fistula,[7] the latter from his knowledge of the pathological anatomy of the "liver of pregnancy," believe that normal pregnancy produces changes (in the liver especially) "difficult to separate from the pathological." [8] They are therefore disposed to look upon departures from non-pregnancy standards in the output of nitrogenous substances in the urine of normally pregnant women as expressions of a functional derangement. But is this necessarily the case? May not such changes in the urinary output of nitrogenous substances be accounted for by perfectly physiological conditions of fetal growth? If so, they cannot without further evidence of a pathological process, be cited as proof of a functional derangement.

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[1] MASSIN: Centralblatt für Gynaekologie, 1895, p. 1105.

[2] ZWEIFEL: Archiv für Gynaekologie, 1904, lxxii, p. 1.

[3] STONE: New York medical record, 1905, lxviii, p. 295.

[4] EDGAR: New York medical journal, 1906, lxxxiii, pp. 897, 956.

[5] EWING and WOLF: American journal of obstetrics, 1907, lv, p. 289.

[6] For a complete review of the literature leading up to this view, *cf.* EWING: American journal of the medical sciences, 1910, cxxxix, p. 828.

[7] "Die schwangere Frau gerade wie ein Thier mit Ecksche Fistel, durch die herabgesetzte oxydation in einen Zustand des Autointoxication versetzt sei," from the Proceedings of the Society for Obstetrics and Gynecology of St. Petersburg, meeting for February 18, 1899, quoted by SCHRADER, *Loc. cit.*

Possible Alterations in the Urine of Pregnancy.

Normal pregnancy has been charged with producing three different sorts of alterations in the metabolic processes, namely:

1. **Deficient power of oxidation.** — (*a*) *Based on low urea.* (Massin,[9] Whitney and Clapp,[10] Edgar.[11]) Zacharjewsky [12] and Schrader [13] did not find the urea nitrogen in normal pregnancy lower than the percentages accepted by them as "normal."

(*b*) *Based on high unoxidized sulphur.* — (Harnack,[14] Schrader,[15] and Hoffström.[16]) Bar [17] found the unoxidized or neutral sulphur increased in case of one dog but diminished in another. In women he found the neutral sulphur quite within normal limits even in the last days of pregnancy.

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[8] *Cf.* opening sentence of Ewing's address before the Pathological Society of Philadelphia, American journal of the medical sciences, *loc. cit.*

[9] Massin: *Loc. cit.* Massin quotes analyses by Poehl (method not given), made probably in 1892 or 1893, which show a lower percentage of urea in the urine of normal pregnancy. Russian literature cited in the Centralblatt article.

[10] Whitney and Clapp: American gynecology, 1903, iii, p. 1. Their urea precipitates "included allantoïn, oxaluric acid, part of the creatinin and oxyproteic acid." The total fraction, however, was reduced in normal pregnancy.

[11] Edgar: *Loc. cit.* Analyses made by Wolf, using Folin's method.

[12] Zacharjewsky: Zeitschrift für Biologie, 1894, xxx, p. 368, using Liebig's method.

[13] Schrader: Archiv für Gynaekologie, 1900, lx, p. 534, using the hypobromite method.

[14] Harnack: quoted by Schrader, *Loc. cit.*

[15] Schrader: using soda and saltpetre fusion method for total sulphur and Salkowski's method for sulphate sulphur.

[16] Hoffström: Skandanavisches Archiv für Physiologie, 1910, xxiii, p.326, using Folin's methods.

[17] Bar: Leçons de pathologie obstétrical, Paris, 1907, p. 556.

2. **Diminished power of deamination.** — (Ewing and Wolf,[18] and Van Hoogenhuyze and ten Doeschate.[19])

Ewing and Wolf, in describing their results on normal women, say: "Comparing these results with our own on healthy men and women and with Folin's analyses of normal urines, the results indicate that in the great majority of cases pregnancy tends to disturb metabolism, lowering the percentage of urea nitrogen and increasing the undetermined or amino acid nitrogen and to a less extent the ammonia." [20] They recognize the fact that these changes according to more modern conceptions do not necessarily depend upon a diminished power of oxidation, and therefore propose the explanation of a diminished power of deamination. "In the breaking down of protein materials during metabolism the step following the conversion of the albumins to simpler compounds, the polypeptids and the amino acids, is the removal of the amino group to form non-nitrogenous compounds, and ammonia. If the amino group is not split off, the amino acids remain. If they are excreted in the urine, as the rise in the undetermined nitrogen and the finding of the individual amino acids in a large series of disorders indicate, it seems probable that these disorders are connected with this deficiency in the organism to remove the amino group and so convert it eventually into ammonia and urea." [21] The Dutch observers adopt the same explanation.

3. **Diminished power of dehydration.** — Van Hoogenhuyze and ten Doeschate offer this explanation for the appearance of creatin in the urine of normally pregnant women who were kept on a creatin-free food.

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[18] Ewing and Wolf: *Loc. cit.* In six cases described as "without symptoms."

[19] Van Hoogenhuyze and ten Doeschate: Annales de gynécologie et d'obstétrique, 1911, xxxviii, p. 17 and p. 97 (in a single primipara), using Folin's methods. In two other cases described as normal they found figures which were approximately "normal" ("*environs des chiffres normaux*").

[20] American journal of obstetrics, 1907, lv, p. 292.

[21] *Idem*, p. 307.

GENERAL CRITICISM OF THE EVIDENCE.

Reproduction by the viviparous method is thus placed under a mild sort of indictment on three different counts. The implication is that nature has adopted a method which *per se* is bad, sexual dimorphism among mammals having proved grossly unfair to the maternal parent, because it imposes conditions which are unphysiological.

In answer to this it might be said in the first place, without resorting to a teleological argument, that there is a strong antecedent presumption against the truthfulness of such a charge. Considered as a general process to which nature has adhered, not for men alone but for all mammals, these millions of years, one would think that any serious defect in the method, as fundamental as a disturbance to the process of oxidation or deamination or dehydration would be, would long since have been eliminated or corrected by adaptation. For, if the defect were of recent origin only, the probability would be that it should be ascribed to the unhygienic conditions of modern life rather than to the method of reproduction itself.

But, in the second place, the evidence is partly bad and partly capable of another interpretation. The analyses which were made before the invention of the newer methods for the determination of urea, ammonia, etc., are not trustworthy so far as absolute figures are concerned. We know that the Liebig method brought down various other nitrogenous constituents than urea, and we know that Salkowski's method for the determination of the oxidized or sulphate sulphur, until its improvement by Folin,[22] gave very variable results, most of which were too low, leaving therefore too much sulphur to be classed as unoxidized or neutral. Nevertheless these methods might still have been used to reveal a difference in composition between urines from pregnant and non-pregnant subjects, *if the other conditions had been carefully controlled.* Here is the main difficulty even where modern and reliable methods have been used: Not one of the observers who report a smaller percentage of urea in the urine of normally pregnant women knew at the time how much nitrogen his subject was ingesting in the foods. Bar is the only investigator who has hitherto reported such determinations in the urine of dogs.

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[22] FOLIN: Journal of biological chemistry, 1905, i, p. 131.

He knew the nitrogen intake and the nitrogen balance. That he did not recognize the true significance of the urinary changes must be ascribed to the fact that he was not abreast of current thought as to the difference between exogenous and endogenous metabolism. If a nitrogen balance had been kept on their pregnant subjects, it certainly would have been apparent to Ewing and Wolf,[23] and probably to other investigators, that *the lowest percentage of urea nitrogen occurs simultaneously with the greatest nitrogen retention.* To take a concrete instance: In Ewing and Wolf's normal case three on August 6th, twenty-four days before delivery, there were 7.36 gm. nitrogen in the urine, 66.7 per cent of which was urea nitrogen, while on November 1st, two months after delivery, there were 11.96 gm., of which 80.3 per cent was urea–N. *It is possible* that the woman ate approximately the same amount of nitrogen on those two days, say 13 gm. On the later date, if she were not nursing the child, which is probably the case, judging merely from the percentage of urea-nitrogen in the urine, she would have been in nitrogen equilibrium. On the earlier date it is likely that she was retaining considerable nitrogen — possibly as much as 4 or 5 gm. daily,[24] so that the 7.36 gm., or so much of it as came from the food, represented simply what was left over after the fetus had taken its quotum. The difference in percentage of urea on the two days, in the writer's opinion, is to be ascribed to the fact that on the former date a portion of the food nitrogen which ordinarily appears in the urine as urea was anabolized on behalf of the fetus, while on the latter date (after parturition and after involution) it was all, or mainly, katabolized by the subject's own body. The same might be said also of their case four, where the amount of nitrogen excreted on the eleventh day before labor was 7.99 gm., of which 53.9 per cent was urea nitrogen, while on the eighteenth day after labor it was 12.84 gm., of which 85.2 per cent was urea nitrogen. In case three the only difference of any consequence so far as absolute quantities are concerned is in the urea nitrogen. The other fractions, especially those which represent the endogenous metabolism, are practically the same before labor as after. In case four the endogenous fractions are slightly higher in absolute figures on the pregnant day, as we should expect them to be, on account of the greater tissue activity.

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[23] Ewing and Wolf appreciated this lack of knowledge, as is evident from the following: "In the interpretation of analyses we must eventually take into consideration the effect of this nitrogen retention on the partition. This is one of the chief factors which, owing to defective knowledge of the metabolism of normal cases, we are as yet unable to estimate." — American journal of obstetrics, 1907, lv, p. 293.

[24] *Cf.* Zacharjewsky's figures and those of Slemons (Johns Hopkins Hospital Report, 1904, xii, p. 111), also those of Hoffström, *Loc. cit.*

What has been said of Ewing and Wolf's figures with regard to urea nitrogen applies with equal force to those of other writers. There can be no doubt of the fact that there is a smaller percentage of urea in the urine of pregnancy, especially in the last two or three months. This has been noted by every observer who has made urea determinations except Schrader and Zacharjewsky, and their failure to confirm it was probably due to faulty methods. Occasionally, as in some of Edgar's cases, the percentage of urea is quite as high as normal. But we know that it is not unusual to find days even in the last weeks of pregnancy when a balance between the intake and output of nitrogen for the twenty-four hours shows no nitrogen retention.[25] It is quite possible that the days of high urea were days of low retention. In fact, if the ideas as to the difference between exogenous and endogenous metabolism introduced by Folin are correct, a high retention of nitrogen could not fail to produce a relatively low percentage of urea nitrogen, and *vice versa*.[26]

But such fluctuations would not denote fluctuations in the oxidative capacity of the organism, even if urea were formed exclusively by a process of oxidation. Neither would they denote fluctuations in the power of deamination of exogenous proteïn. If this were the case there would be an absolute swelling of the undetermined or rest nitrogen with every absolute shrinkage of the urea nitrogen and *vice versa;* that is, if the food contained the same quantity of nitrogen. Unfortunately we do not know how much nitrogen the food contained in any of the reported normal cases where urea was determined; but at all events there is no satisfactory evidence that the nitrogen which failed to appear as urea did appear as undeaminized nitrogen. In most instances where the undetermined nitrogen is higher, it is only relatively so, and this relative increase is shared by the ammonia, creatinin, and uric acid nitrogen. In conclusion of this matter for the present it must be noted that one cannot judge the power of the organism to deaminize the protein cleavage products without knowing how much protein has a chance of being deaminized. In pregnancy the fetus draws its supply of protein from the blood plasma, and when this depleted plasma passes through the intestinal wall it may be supposed to cause there and then, if protein cleavage products (not necessarily amino acids, but polypeptids as well) are available, a resynthesis which makes good the depletion.[27] Such protein never would have a chance of being deaminized, for its nitrogen would never be split off as ammonia, and consequently the ammonia as carbonate or carbamate would not be hydrolyzed to urea.

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[25] *Cf.* ZACHARJEWSKY's tables, also those of SLEMONS, *Loc. cit.*

[26] This relationship is exemplified in the period of recovery following a fasting period in the work of HOWE, MATTILL and HAWK (Journal of the American Chemical Society, 1911, xxxiii, p. 568) and in the work of CATHCART (Biochemische Zeitschrift, 1907, vi, p. 109). It would appear also from CATHCART's tables that the nitrogen held back by the use of starch and cream diet was mainly urea and ammonia nitrogen potentially, and the sulphur held back was mainly sulphur which in complete inanition was oxidized.

The evidence of diminished oxidation is no better when we consider the unoxidized or neutral sulphur in proportion to the total sulphur. Folin[28] classed the inorganic sulphate sulphur as belonging to the exogenous metabolism; that is, it varies in absolute amount with the total amount of nitrogen (and sulphur) in the food and therefore in the urine. It is mainly this sulphur, therefore, which would be held back from the food to make good any deficiency such as that caused by the fetus, and we should expect to find it present in lower absolute, and lower relative amount, especially in the latter part of the pregnancy. That this is the case is shown in the excellent work of Hoffström.[29] This author followed the sulphur metabolism from the seventeenth week to the end of pregnancy in his single subject. He determined the oxidized sulphur, including both the inorganic-sulphate sulphur and the ethereal sulphate sulphur by the Folin method[30] and the total sulphur by the sodium peroxide method. The average percentage of the neutral sulphur for this long period was 33.4 per cent of the total. Hoffström compares this figure with that given by Van Noorden (14 to 20 per cent) as normal, and concludes that "the pregnant organism is unable to completely oxidize the products of disassimilation." His table, however, shows no absolute increase in the quantity of unoxidized sulphur as pregnancy advances. The relative increase is due solely to the relative decrease in the amount of oxidized sulphur. Since Hoffström had determined the sulphur balance also and his curve shows in general a greater retention of this element where the oxidized sulphur in the urine is least, it is strange that he should have rejected this obvious explanation of the high neutral sulphur, as he deliberately does, for the old idea of diminished power of oxidation. If, as seems possible, Hoffström's "oxidized sulphur" really included only the inorganic-sulphate sulphur (see note 30), his figure of 33.4 per cent would include both neutral and ethereal sulphur, and this would not be much beyond "normal" limits. As a matter of fact perfectly normal figures for the neutral or unoxidized sulphur have been frequently found. In several of Schrader's cases the percentage was well within the limits given by Van Noorden and some were considerably below.[31] Bar[32] found only 9.05 per cent as an average for some six or eight cases. Such figures would mean that unoxidized sulphur is retained in greater quantity than the oxidized sulphur.

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[27] *Cf.* Abderhalden and Samuely: Zeitschrift für physiologische Chemie, 1905, xlvi, p. 193.

[28] Folin: This journal, 1905, xiii, pp. 66, 117.

[29] Hoffström: *Loc. cit.*

[30] *Loc. cit.* p. 375. It is not clear, however, from Hoffström's description of his method (*Loc cit.*, p. 347) that the oxidized sulphur includes anything more than the inorganic-sulphate sulphur: "das oxydierte S (war) so bestimmt dass der Harn (50 c.c.) nach Ansäuerung mit Salzsäure mit Barium chlorid ausgefällt wurde."

This is the whole case for diminished oxidation. There is no collateral evidence. Observations by Carpenter and Murlin[33] on the total absorption of oxygen, and the total energy production in three normal cases of pregnancy have shown that the total oxidation in the last weeks of pregnancy was slightly higher, measured per unit of weight, than was the average for eight women in complete sexual rest. The figures of Bar (on women) above cited and some of those of Schrader seem also to bear out the idea that oxidation instead of being lower is really higher in pregnancy.

The third point, namely, that of diminished power of dehydration, we shall return to later.

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[31] SCHRADER: *Loc. cit.*

[32] BAR: *Loc. cit.*, p. 556.

[33] CARPENTER and MURLIN: Archives of internal medicine, 1911, vii, p. 184.

AUTHOR'S EXPERIMENTS.

Positive support for the explanation of the urinary alterations in normal pregnancy suggested above can only be had from experiments in which the nitrogen intake is known; and the evidence will be convincing only if the same subject is used and if the intake of nitrogen is the same, or nearly so, in the pregnant as in the non-pregnant condition.

Since the question involved is a broad biological one, the evidence should be obtained from other mammalian species as well as man. The kinds of materials required for development of the fetus must be very nearly the same in all mammalian species, and the quantities required, considered in relation to the mass of the mother's body are not so widely different, in the different domestic species at least, as might be expected, especially when the entire birth is considered.[34]

The observations on dogs about to be reported have been made in the course of the past three years. Three of the experiments (I, IV, and V) were planned for the purpose of following the nitrogen balance in pregnancy and menstruation and have been partially reported as Experiments I, II, and III in the second paper of this series.[35] Experiments I and II of this paper were also the basis of the observations on the energy metabolism of pregnancy reported in the first paper of the series.[36] Experiments I, II, and V were made on the same dog; III on a second, and IV on a third.

The protocols will not be repeated here in detail. Any one wishing further information will find it in the preceding papers. The following facts, however, will be necessary to an understanding of the tables, which are numbered in the same order as the experiments.

Experiment I. Dog A. First Pregnancy. — Copulation on April 22, 1908. A single puppy was born on June 26th. Detailed analyses were made only on the last two days of each week, when the dog was in the respiration apparatus (see Experiment I of paper I). Food during the last five weeks consisted of beef heart, crackermeal, lard, bone ash, and salt, and contained 8.9 gm. of N and 902 calories of energy.

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[34] *Cf.* RUBNER: Archiv für Hygiene, 1908, lxvi, p. 127.

[35] MURLIN: This journal, 1910, xxvii, p. 177.

[36] MURLIN: *Ibid.*, xxvi, p. 134.

Experiment II. Dog A. Second Pregnancy. — Copulation on October 10, 11, and 12, 1908. Five puppies born on December 14th. Food during the early part of the experiment was creatin-free, consisting of 50 gm. plasmon, 40 gm. lard, 60 gm. cane sugar, 5 gm. bone ash and 2 gm. of salt and containing 6.0 gm. N and 800 cal. From November 7th to the end of the experiment (with the exception of a few days in the VIIth week of pregnancy when the creatin-free diet was given) the food was as nearly as possible the same as in Experiment I. The urine was saved for analysis only on the days mentioned in the table. Care was taken, however, to save no urines immediately after a change in the diet.

Experiment III. Dog B. — This dog was a pet borrowed for the purpose of the experiment. Observations were made only at the beginning and the end of the pregnancy. The dog was brought into the laboratory a few days previous to copulation, which occurred on October 6 and 7, 1908, and was placed on a creatin-free food similar in all respects to that given above for Experiment II, but containing 5.95 gm. N and 950 cal. The dog was dismissed on October 15th, but was returned to the laboratory on November 28th, when the original diet was resumed. Parturition occurred December 3d, eight puppies being born on the 57th day of gestation. The urine was collected only on the days mentioned in Table III.

Experiment IV. Dog C.[37] — Copulation on February 1, 2, and 3, 1909. Four puppies born on April 4th. Food, same in general character as in Experiment I, but contained 8.0 gm. N and 993 cal. Urine collected in weekly periods. (For further details see Experiment II of paper II.)

Experiment V. Dog A. Third Pregnancy. — The object of this experiment was to compare the metabolism of entire sexual rest with that of menstruation and early pregnancy. Copulation occurred on April 23, 1909. Four puppies were born on June 26th. (Compare dates of first pregnancy the previous year). Diet throughout exactly the same as in the last five weeks of the preceding pregnancies. Urine collected in weekly periods. (Experiment III, II paper.)

Plan and Scope.

When the work was begun, it was planned to make the analyses of both urines and feces as complete as possible all the way through the pregnancy and to compare these results with others obtained from the same dogs kept on the identical diets when not pregnant. This ideal has been only partially realized. In the first experiment the total P_2O_5 and the total Cl[38] were determined, but in the later experiments, partly for want of time and partly because these latter constituents were evidently not so important for the purpose of testing the qualitative effects of pregnancy, the analyses were limited to the nitrogen and sulphur fractions, or, as in Experiment III, to the nitrogens alone.

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[37] This was Dog B of the second paper.

All the analyses were made while the urines were fresh or within a few weeks of their collection, toluol or thymol-chloroform and refrigeration being used meantime to insure their preservation. Publication has been delayed purposely to make the series more complete by observations on other dogs fed in different ways. Several recent attempts to induce dogs to breed in the laboratory having, however, proved unsuccessful, and the work already accomplished having furnished positive evidence on a number of points, it seems undesirable to delay the report further.

METHODS.

The methods used were as follows: Total nitrogen by Kjeldahl method, using Folin's copper sulphate and potassium sulphate mixture as catalyzer; urea by Folin's method, a few by Mörner-Sjökwist method for comparison; ammonia by Shaffer's method in Experiment I, in all the others by Folin's method; creatin and creatinin by the colorimeter method of Folin; total sulphur by Osborne's sodium peroxide method; inorganic and ethereal sulphate by Folin's methods; total P_2O_5 by uranyl-acetate method and chlorides by Volhard's method. Tests for albumin were regularly made by means of the acetic acid-potassium ferrocyanide method and for indican by use of Obermyer's method.

RESULTS.

The first experiment of this series was almost ideal, at least for several weeks, so far as the control of external conditions was concerned. Not only was the quantity and the quality of the food exactly the same every day from the beginning of the fifth week to the end, and not only was the dog kept in the same cage, permitting thus about the same amount of muscular exercise from day to day, but for four weeks, the fifth to eighth inclusive, it happened that the cage was exposed to the same temperature (within less than 2° C.) on the days when the urines were collected. While it is known that neither the muscular activity nor the external temperature influences the total output of nitrogen to any great extent, there are a few experiments in the literature[39] which make it appear advisable in experiments of this sort to have all the external conditions as nearly uniform as possible. In the other experiments this idea was kept constantly in mind, though the temperature varied more than in the first. Any change either in the total nitrogen or in the partition of the nitrogen from week to week during this period must then be due to the processes going on in the uterus. It has been pointed out in the second paper of this series that the quantity of nitrogen stored in the embryo up to the end of the fourth week is negligible. In fact, from data there gathered together from all the work which has been done on animals, it was concluded that there is a strong tendency for the mother's body to be in minus nitrogen balance up to this time. What effect this extra loss of nitrogen from the mother's body would have cannot be ascertained from the first four experiments because on the one hand in none of these was the experiment started long enough before the beginning of gestation to afford any basis of comparison with the pre-œstrual period, and on the other the quantity and quality of the food was not constant enough from the very first of gestation to afford a proper basis of comparison with the later stages of pregnancy. In the fifth experiment, however, where the diet was constant from the fifth week previous to copulation and the analyses were made from the third week previous to the second week following inclusive, some effects of this tendency to lose nitrogen can be seen in the nitrogen partition (Table V).

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[38] The writer is indebted to Dr. A. C. Reed for many of the analyses in this first experiment.

Because this experiment gives us a picture of the metabolism in sexual rest which can serve as a basis of comparison for the other experiments on this same dog, we shall consider it first. In the week designated I, in reality the third of this diet, the dog was retaining an average of 0.28 gm. N each day (last column of

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[39] *Cf.* Voit: Zeitschrift für Biologie, 1878, xiv, p. 80, and Shaffer: This journal, 1908, xxii, p. 445. Shaffer's experiments show a small but constant increase in the percentage of oxidized sulphur in the urine after muscular exercise.

TABLE I.

EXPERIMENT I. DOG A. NITROGEN PARTITION. SULPHUR PARTITION. TOTAL P_2O_5 EACH WEEK OF PREGNANCY. UPPER FIGURES GRAMS.

Month and day.	Week.	Wt. kgm.	Food. Average per day.			Nitrogen fractions.				
			Total cal.	Cal. per kgm.	N in food.	Total N.	Urea N.	NH_3 N.	Urea + NH_3 N.	Creatinin N.
1908 April 30-May 1[2]	I.	12.29	662.5	53.8	7.69	6.65	6.02	0.32	6.34	0.108
							90.4	**4.8**	**95.2**	**1.6**
May 7–8	II.	12.24	905.0	73.9	8.36	7.61	6.73	0.43	7.16	0.103
							88.5	**5.7**	**94.0**	**1.4**
May 15–16	III.	12.63	867.2	68.8	8.25	7.64	6.45	0.42	6.88	0.112
							84.7	**5.4**	**90.1**	**1.5**
May 21–22	IV.	12.71	867.2	68.3	8.25	7.76	6.76	0.41	7.17	0.130
							87.2	**5.3**	**92.5**	**1.7**
May 28–29	V.	13.07	907.4	69.3	8.99	8.20	7.22	0.34	7.56	0.129
							88.0	**4.1**	**92.1**	**1.6**
June 4–5	VI.	13.33	907.4	68.1	8.99	7.93	6.91	0.35	7.26	0.132
							87.2	**4.4**	**91.6**	**1.7**
June 11–12	VII.	13.80	907.4	66.4	8.99	7.95	...	...	...	...
							...	...	...	...
June 18–19	VIII.	14.04	907.4	64.4	8.99	7.17	6.13	0.34	6.46	0.130
							85.3	**4.7**	**90.0**	**1.8**
June 24–25	IX.[5]	14.40	907.4	62.5	8.99	7.58	6.61	0.28	6.89	0.124
							87.2	**3.7**	**90.9**	**1.6**
July 1–2	Post-part. I.[1]	13.98	907.4	64.9	8.99	8.86	7.80	0.33	8.13	0.127
							88.0	**3.7**	**91.7**	**1.4**
July 15[3]	Post-part.	13.78	907.4	65.0	8.99	4.58[6]	3.89	0.26	4.15	0.134
							85.1	**5.7**	**90.8**	**2.9**

[1] For first three weeks dog was fed 1 gm. NaCl each day; from fourth week on 2 gm. [2] At end of week. [3] Two weeks after lactation had ceased.

TABLE I.

AND CHLORINE. QUANTITIES IN GRAMS PER DAY. AVERAGE FOR LAST TWO DAYS OF
LOWER FIGURES PERCENTAGES OF TOTAL.

		Sulphur fractions.						Nitrogen balance.	
Creatin N.	Undetermined N.	Total SO_3.	Inorganic SO_3.	Ethereal SO_3.	Neutral SO_3.	Total P_2O_5.	Cl.	By weeks.	Av. Per day.
0.013	0.191	...	0.684	0.072	0.178	0.50	1.15	−0.975	...
0.2	**1.9**	0.934	**73.3**	**7.7**	**19.0**				
0.013	0.320	...	0.668	0.060	0.238	0.46	0.90	−2.436	...
0.2	**4.1**	0.966	**69.2**	**6.2**	**24.8**				
0.155	0.493	...	0.994	0.096	0.198	0.98	0.67 [1]	−2.289	...
2.0	**6.4**	1.29	**77.2**	**7.4**	**15.4**				
0.186	0.270	...	0.910	0.096	0.232	1.285	1.07	−0.325	...
2.4	**3.4**	1.238	**74.3**	**7.9**	**17.8**				
0.180	0.335	...	0.97	0.08	0.32	1.13	1.61 [4]	+3.153	0.45
2.2	**4.1**	1.38	**70.3**	**6.2**	**23.4**				
0.211	0.329	...	0.86	0.06	0.38	1.25	1.58	+3.048	0.43
2.7	**4.0**	1.30	**66.2**	**4.8**	**29.0**				
...	...	...	0.95	0.06	0.28	1.11	1.53	+5.614	0.80
...	...	1.30	**73.1**	**4.9**	**22.0**				
0.229	0.344	...	0.80	0.05	0.296	1.14	1.54	+5.714	0.81
3.2	**4.8**	1.15	**70.0**	**4.3**	**25.7**				
0.259	0.313	...	0.84	0.08	0.21	1.01	1.54	+6.613	0.94
3.4	**4.1**	1.14	**74.2**	**7.5**	**18.3**				
0.194	0.413	...	1.00	0.08	0.33	1.26	1.81	−0.593	...
2.2	**4.7**	1.42	**70.7**	**6.1**	**23.2**				
0.106	0.183	...	0.46	0.06	0.13	0.81	1.86	....	...
2.1	**4.2**	0.64	**71.4**	**8.7**	**19.5**				

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[4] From fifth week on food was exactly the same each day.

[5] Parturition on June 26.

[6] Probably some urine lost.

TABLE II.

Dog A. Second Pregnancy. Nitrogen and Sulphur Partitions in Daily

Day.	Weight kgm.	Food.		Analysis				
		Cal. per kgm.	N in food.	Reaction.	Albumin.	Nitrogen.		
						Total N.	Urea + NH_3 N.	Creatinin N.
1908 Oct. 5[1]	12.94	65.7	6.18	ac.	+	4.74	4.42 **93.3**	0.118 **2.4**
Oct. 10[2]	12.86	66.0	6.00	alk.	–	4.78	4.35 **91.1**	0.108 **2.4**
Oct. 14	12.86	62.3	6.00	alk.	–	4.85	4.42 **91.4**	0.115 **2.3**
Oct. 31	13.70	59.0	6.00	alk.	–	4.96	4.57 **92.2**	0.118 **2.4**
Nov. 7[3]	13.90	64.5	8.99	ac.	–	8.75	7.96 **91.2**	0.137 **1.5**
Nov. 14[4]	14.20	64.0	8.99	ac.	–	8.16	7.46 **91.4**	0.118 **1.5**
Nov. 21[5]	14.70	61.0	8.99	ac.	–	7.96	7.39 **92.6**	0.129 **1.6**
Dec. 8[6]	16.00	54.7	8.99	ac.	–	5.60	4.99 **89.0**	0.133 **2.4**
Dec. 9	16.36	55.4	8.99	ac.	–	5.71	4.89 **85.7**	0.148 **2.6**
Dec. 10	16.52	54.9	8.99	ac.	–	6.03	5.21 **85.5**	0.135 **2.3**
Dec. 11	16.86	53.7	8.99	ac.	+	6.28	5.35 **86.4**	0.144 **2.2**
Dec. 16	14.08	64.4	8.99	ac.	–	7.89	7.14 **90.5**	0.146 **1.9**
Dec. 17	13.92	64.7	8.99	ac.	–	6.85	6.21 **90.5**	0.147 **2.2**

[1] Fifth day before copulation.

[2] First day of copulation.

TABLE II.

URINES. UPPER FIGURES GRAMS. LOWER FIGURES PERCENTAGE OF TOTAL.

OF URINE.						Remarks.
Nitrogen.		Sulphur.				
Creatin N.	Undetermined N.	Total SO_3.	Inorganic SO_3.	Ethereal SO_3.	Neutral SO_3.	
0.0	0.20 **4.3**	. . .	. . .	. . .	. . .	Creatin-free food.
0.0	0.32 **6.5**	. . .	. . .	. . .	. . .	Creatin-free food.
0.008	0.30 **6.2**	. . . 0.55	0.39 **71.8**	0.013 **2.3**	0.14 **25.9**	Creatin-free food.
0.0	0.27 **5.4**	. . .	. . .	. . .	. . .	Creatin-free food.
0.27 **2.5**	0.42 **4.8**	. . . 1.29	1.04 **80.5**	0.07 **5.2**	0.18 **14.3**	Food con'd 0.3 gm. creatin N (Mellanby's method).
0.15 **1.9**	0.42 **5.2**	. . .	. . .	. . .	. . .	
0.18 **2.4**	0.25 **3.4**	. . .	. . .	. . .	. . .	
0.21 **3.9**	0.25 **4.7**	. . . 0.67	0.49 **72.3**	0.07 **10.0**	0.12 **17.7**	Food con'd 0.3 gm. creatin N (Mellanby's method).
0.21 **3.8**	0.46 **7.9**	. . .	. . .	. . .	. . .	
0.36 **5.7**	0.32 **6.5**	. . .	. . .	. . .	. . .	
0.35 **6.0**	0.43 **5.4**	. . . 0.87	0.45 **51.6**	0.19 **21.9**	0.23 **26.5**	Parturition on Dec. 14. Five puppies born.
0.30 **3.8**	0.30 **3.8**	. . . 0.97	0.76 **78.1**	0.06 **6.4**	0.15 **15.7**	
0.35 **5.2**	0.15 **2.1**	. . . 0.89	0.71 **79.5**	0.04 **4.8**	0.14 **15.5**	

[3] Fourth week. [4] Fifth week. [5] Sixth week. [6] Eighth week.

TABLE III.

EXPERIMENT III. DOG B. (ON CREATIN-FREE FOOD.)

Day.	Weight kgm.	Food.		Analysis of urine.				
		Cal. per kgm.	N in food.	Total N.	Urea + NH_3 N.	Creatinin N.	Creatin N.	Undet. N.
1908 Oct. 9[1]	14.00	54.8	5.950	4.425	3.929	0.118	0.016	0.362
					89.0[2]	**2.7**	**0.3**	**8.0**
Oct. 10	14.00	54.8	5.950	4.672	4.672	0.103	0.011	0.346
					90.0	**2.2**	**0.2**	**7.6**
Oct. 11	14.00	54.8	5.950	4.890	4.498	0.118	0.0	0.274
					91.7	**2.4**	...	**5.9**
Oct. 14	13.92	68.7	5.950	5.355	4.853	0.124	0.0	0.376
					91.0	**2.3**	...	**6.7**
Dec. 1	16.86	56.9	5.950	4.391	3.962	0.137	0.013	0.278
					90.5	**3.1**	**0.3**	**6.1**
Dec. 2	16.90	62.0	5.950	3.605	3.248	0.137	0.016	0.204
					90.3	**3.8**	**0.4**	**5.5**
Dec. 3 (Day of part.)	16.87	60.4	4.876	5.997	5.112	0.135	0.101	0.649
					85.7	**2.2**	**1.7**	**10.4**
Dec. 4	13.93	75.4	5.950	4.533	3.891	0.133	0.042	0.467
					86.0	**2.9**	**0.9**	**10.2**
Dec. 8	12.60	90.6	4.876	5.069	4.319	0.129	0.137	0.484
					85.3	**2.5**	**2.4**	**9.5**
Dec. 10	12.75	95.0	5.836	3.855	3.213	0.141	0.063	0.438
					83.6	**3.6**	**1.6**	**11.2**

[1] Copulation Oct. 6 and 7. [2] Upper figures grams; lower figures percentage of total N.

table). The urea + ammonia nitrogen amounts to 92.3 per cent of the total, and the undetermined or rest nitrogen to 2.3 per cent. The inorganic-sulphate sulphur, expressed in all these tables as SO_3, amounts to 75.6 per cent of the total and the neutral or unoxidized sulphur to 21.5 per cent. The next week shows some sign (in the higher nitrogen retention) of the approaching menstruation (see second paper); but the nitrogen and sulphur partitions are but little altered. First signs of menstrual blood were obtained on April 16th, and during the week which followed the menstrual hemorrhage was profuse. Copulation occurred on the 23d and the bleeding ceased about three days later. The effect of this nitrogen retention, which probably serves to compensate the loss by hemorrhage, is to decrease the absolute quantity of urea + ammonia nitrogen in about the same degree; that is, the percentage of the total remains the same. The creatinin nitrogen is absolutely the same, while the creatin nitrogen is slightly and the undetermined nitrogen is plainly reduced. The only percentage difference of any consequence is that of the undetermined nitrogen. The oxidized sulphur is less in proportion to the entire output of sulphur during this week, and the unoxidized is higher.

After copulation the urea + ammonia nitrogen goes down relatively, the undetermined goes up, while the creatinin and creatin fractions remain constant. The oxidized sulphur remains lower relatively after menstruation and copulation than before, and the unoxidized remains higher. The high unoxidized sulphur during the week of menstruation (active bleeding) is probably due to retention of sulphur which ordinarily would be oxidized, while the lower undetermined nitrogen may indicate a retention of endogenous products. However this may be, it is difficult to escape the belief that after gestation both the nitrogen and sulphur partitions are influenced by a more active endogenous metabolism, as compared with the weeks previous to menstruation. The urinary picture is not inconsistent with this view, which has already been presented in the previous paper (see also page 450).

Returning to Experiment I and comparing the nitrogen partitions from week to week from the point at which the diet and all external conditions became constant (fifth week), it is evident that there is a progressive diminution in the output of urea nitrogen which runs parallel with the nitrogen retention (compare total N of urine with N of food). The highest retention on the days of analysis was in the

TABLE IV.

EXPERIMENT IV. DOG C. URINES COLLECTED IN

Week of pregnancy.	Food.		Nitrogen fractions in urine. Upper figures grams; lower figures percent-				
	Calories per kgm.	Total N.	Total N.	Urea N.	NH_3 N.	Urea + NH_3 N.	Creatinin N.
1909 I . . . . (Feb. 3–9.)	56.0	7.44	8.43	7.38	0.34	7.72	0.19
				87.6	**4.0**	**91.6**	**2.7**
II . . . (Feb. 10–16.)	72.0	8.00	7.95	6.61	0.33	6.94	0.19
				83.3	**4.2**	**87.5**	**2.4**
III . . .	71.0	8.00	8.17	7.09	0.30	7.39	0.19
				86.8	**3.7**	**90.5**	**2.3**
IV . . .	71.0	8.00	8.56	7.43	0.32	7.75	0.19
				86.8	**3.8**	**90.6**	**2.2**
V . . .	70.0	8.00	8.36	7.26	0.31	7.56	0.19
				87.0	**3.7**	**90.7**	**2.3**
VI . . .	68.0	8.00	7.92	6.93	0.31	7.23	0.18
				87.5	**3.9**	**91.4**	**2.3**
VII . .	66.0	8.00	7.21	6.12	0.35	7.46	0.18
				85.0	**4.8**	**89.8**	**2.6**
VIII . .	63.0	8.00	6.08	5.15	0.28	5.43	0.17
				85.0	**4.6**	**89.6**	**2.7**
IX . . . (March 31–Apr. 3.)	61.0	8.01	5.81	4.92	0.21	5.13	0.15
				84.6	**3.5**	**88.1**	**2.6**
Post-part.[1] V . . . (May 7–12.)[2]	76.0	8.01	6.68	5.74	0.33	6.07	0.16
				86.0	**4.9**	**90.9**	**2.4**

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[1] Parturition April 4.

TABLE IV.

WEEKLY PERIODS. FIGURES IN GRAMS PER DAY.

age of total N.		Sulphur fractions. Upper figures grams; lower figures percentage of total S.				Nitrogen balance.	
Creatin N.	Undetermined N.	Total SO_3	Inorganic SO_3.	Ethereal SO_3.	Neutral SO_3.	By weeks.	By day.
0.16	0.35	...	...	...	...	−8.824	−1.26
1.9	**4.2**						
0.23	0.59	...	...	...	...	−4.830	−0.69
2.9	**7.5**						
0.20	0.36	...	...	...	...	−5.968	−0.85
2.5	**4.4**						
0.19	0.43	1.42	0.99	0.07	0.37	−8.445	−1.20
2.3	**5.1**		**69.7**	**4.7**	**25.5**		
0.22	0.36	1.33	0.97	0.04	0.31	−6.531	−0.94
2.6	**4.4**		**73.3**	**3.1**	**23.5**		
0.20	0.27	1.58	1.16	0.04	0.37	−4.001	−0.59
2.5	**3.4**		**73.5**	**2.3**	**23.2**		
0.21	0.35	1.19	0.82	0.05	0.32	+1.801	+0.25
2.9	**4.8**		**69.0**	**3.8**	**27.2**		
0.24	0.24	0.89	0.59	0.03	0.26	+9.021	+1.26
4.0	**3.9**		**67.0**	**3.9**	**29.1**		
0.26	0.27	0.79	0.52	0.04	0.23	+6.250 (4 days)	+1.56
4.4	**4.7**		**65.8**	**5.2**	**29.0**		
0.19	0.24	1.10	0.76	0.05	0.29	+8.048	+1.15
2.9	**3.7**		**68.7**	**5.0**	**26.3**		

---

[2] Two weeks after lactation had ceased.

TABLE V.

EXPERIMENT V. DOG A. THIRD PREGNANCY. NITROGEN AND SULPHUR PARTITIONS IN

Date.	Week of exp.	Weight kgm.	Food.		Nitrogen.			
			Cal. per kgm.	N in food.	Total N.	Urea + NH_3-N.	Crea-tinin N.	Crea-tin N.
1910 April 3–9	I.	12.94	70	8.99	8.16	7.54	0.15	0.30
						92.3	**1.7**	**3.7**
April 10–16	II.	13.1	69	8.99	7.88	7.20	0.15	0.30
						91.4	**1.9**	**3.8**
April 17–23[1]	III.	13.3	68	8.99	7.44	6.90	0.15	0.26
						92.5	**2.0**	**3.6**
April 24–30	IV.[2]	13.5	66	8.99	7.92	7.17	0.15	0.30
						90.6	**1.9**	**3.8**
May 1–7	V.[3]	13.58	65	8.99	8.40	7.61	0.16	0.29
						90.8	**1.9**	**3.5**

[1] Week of menstruation.

[2] First of

eighth week, and this marks at the same time the lowest quantity of urea both relatively and absolutely. Comparing the other fractions for the fifth and eighth weeks, we see that the ammonia nitrogen and the creatinin nitrogen are the same in absolute figures, but are relatively a little higher in the eighth. The creatin and undetermined nitrogen are higher in both senses. In the ninth week the retention of nitrogen was not quite so high (the last two days before delivery), and consequently the urea is not quite so low.

The sulphur fractions in this experiment do not exhibit the effect of the retention to the same extent.

In Experiment II the same tendency is seen from November 7th, the time at which the food became constant, to the end. That is, the urea nitrogen goes down more rapidly than the total urinary nitrogen, while the creatin and undetermined nitrogen go up, the former in absolute as well as relative amounts and the latter in relative

TABLE V.

WEEKLY URINES. UPPER FIGURES GRAMS. LOWER FIGURES PERCENTAGES OF TOTAL.

	Sulphur.				Nitrogen balance.		Remarks.
Undetermined N.	Total SO_3.	Inorganic SO_3.	Ether SO_3.	Neutr. SO_3.	By weeks.	Per day.	
0.17	1.08	0.82	0.03	0.23	+1.96	+0.28	....
2.3		**75.6**	**2.9**	**21.5**			
0.23	1.22	0.94	0.05	0.25	+4.23	+0.60	....
2.9		**77.2**	**2.0**	**20.8**			
0.13	1.20	0.84	0.03	0.33	+6.37	+0.91	First signs of menstrual blood on April 16.
1.8		**69.7**	**2.8**	**27.5**			
0.29	1.31	0.93	0.06	0.32	+4.15	+0.59	First copulation on April 23.
3.7		**71.0**	**4.6**	**24.4**			
0.33	1.45	1.03	0.05	0.36	+1.51	+0.22	....
3.8		**71.5**	**3.5**	**25.0**			

pregnancy.

[3] Second of pregnancy.

amounts only (*cf. e. g.* December 11th, three days before parturition, with November 7th). The sulphur fractions, so far as the analyses were made, show exactly the same relationship. The inorganic-sulphate sulphur falls both relatively and absolutely as the nitrogen retention becomes greater, and the neutral sulphur rises, though it does not rise much in absolute amount owing to the increase in etherial-sulphate sulphur.

In Experiment IV the law, for such it seems to be, is still better exemplified. The exogenous fractions both of nitrogen and sulphur become both absolutely and relatively less as pregnancy advances, while the endogenous fractions become only relatively greater (creatinin and unoxidized sulphur). The creatin in this dog became both relatively and absolutely greater, as in Experiment I and II, but the undetermined nitrogen became less absolutely and remained stationary in the relative sense (*cf. e. g.* fifth and ninth weeks).

The three experiments which cover the latter half of pregnancy, with the pregnant animal on a constant diet for this time, are in substantial agreement and furnish, it seems, material support for the hypothesis advanced in a previous section (page 426); namely, that low urea nitrogen and high unoxidized sulphur are due mainly, if not solely, to the retention of proteins by the product of conception. On this hypothesis we should expect that the reduction of the urea and the oxidized sulphur would be greater the larger the product of conception, *i. e.*, the larger the number of puppies born. At the same time we should not expect the reduction in percentage to be exactly proportional to the number of puppies born, for a portion of the substances retained must be held back by the uterus itself; and it is perfectly well known that the uterus does not hypertrophy five times as much for five puppies as it does for one. Taking the urea and NH_3 nitrogen together, for both represent deamidized nitrogen, in the first pregnancy of Dog A, from which only a single puppy was born, there is a reduction from the fifth to the eighth week of 2.1 per cent. In the second pregnancy, from which five puppies were born, the greatest reduction between corresponding points (November 14th and December 8th) is 2.4 per cent. But counting to the 9th of December it is 5.7 per cent, and to the 10th, 5.9 per cent, or, at most, only three times as much as in the first pregnancy. In the pregnancy of Dog C, from which four puppies were born, the food contained about 1 gm. less nitrogen every day, although the dog was a kilogram or more heavier than Dog A. (there was no plus balance until the seventh week; see last column of table). The retention from the food therefore is not so rapid as in the second pregnancy of Dog A. The greatest percentage reduction due to retention (from the fifth week to the ninth) is only 2.6 per cent.

THE CREATIN NITROGEN.

This fraction seems to call for a special discussion. Creatin appears in the urine either when there is considerable creatin in the food, or when active destruction of muscular tissue is going on.[40]

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[40] *Cf.* KLERCKER: Biochemische Zeitschrift, 1907, iii, p. 45; MELLANBY: Journal of physiology, 1908, xxxvi, p. 447; SHAFFER: This journal, 1908, xxiii, p. 1; and LEVENE and KRISTELLER: This journal, 1909, xxiv, p. 45.

In Experiments I, IV, and V there was creatin in the food throughout the experiment. In Experiment II the food for the first three weeks was creatin-free (see protocol) and in Experiment III it was creatin-free as long as the dog was under experiment. Experiment III shows a small quantity of creatin in the urine just after copulation and again just before parturition. The quantity is too small to attach any great significance to it. But there is a significant increase in the quantity of creatin just before parturition in all the other experiments (I, II, and IV). The quantity in the food was presumably constant, since the beef hearts which were fed from day to day were fairly uniform in size. In Experiment II the quantity of creatin in the beef heart fed for one day was determined by Mellanby's method[41] on two different days, and proved to be about 0.3 gm., estimated as nitrogen. Up to December 9th, five days before parturition, there was less creatin nitrogen in the urine than was fed; but on the 10th and 11th, and presumably on the next three days, there was more. In Experiment I, where the urine was analyzed only on the last two days of each week and where the amount of creatin fed may be supposed to have been the same as in Experiment II, since the amount of meat was the same, the quantity excreted was never equal to the quantity fed, although it rises almost to this point in the last week. In Experiment IV, where the urine was analyzed only in weekly periods and the quantity of creatin nitrogen present in the food would be (assuming the same percentage in the beef heart) about 0.27 gm., the same relationship is seen. The quantity excreted approaches that in the food only in the last week.

There are only two possible ways of explaining this increase in the creatin nitrogen — one, as given by Van Hoogenhuyze and ten Doeschate (*Loc. cit.*) for exactly similar phenomena in pregnant women, that the liver has suffered a diminution of its dehydrating power by which the preformed creatin is converted to creatinin and the other, that an extra quantity of creatin is being formed in the maternal body either as the result of tissue changes in the uterus before labor is inaugurated, or as the result of inanition. To take the last supposition first, if creatin is formed in the body, it certainly

[41] MELLANBY: *Loc. cit.*

is not the result of insufficient food energy, at least so far as Experiments I and II are concerned, for the energy production of the dog was estimated from the output of carbon and nitrogen during the last days of both these pregnancies (see paper I),[42] and at no time did the energy production reach the level of the energy intake by 20 per cent. It might arise, however, as a result of inadequate protein supply. If the protein fed was insufficient in amount, or if it was of such a nature that it was digested too readily, being thereby supplied to the maternal blood (for a time) more rapidly than it could be synthesized by the product of conception, the result would be its removal as urea, etc., within the early hours (food given all at one meal), and a consequent condition of relative protein starvation for the later hours of the day. During these later hours then there might be a compensatory break down of body musculature yielding creatin as in total starvation.

An experiment has already been reported in the second paper of this series, which tends to support this possibility. The subject was Dog A, and the food was the same in every respect as in Experiment I and II. In two twenty-four-hour periods during the last week of pregnancy, the urine was collected in three-hour periods following a single feeding. On both days the total quantity of nitrogen in the urine was about 5.9 gm., of which 3.9 gm. the first day and 3.8 the second were excreted the first twelve hours, leaving 2.0 gm. and 2.1 gm. respectively to be excreted the second twelve hours. The appearance of the curve suggests that this 2 gm. might well have been largely endogenous nitrogen, and, as indicated above, a part of it might be endogenous creatin. Unfortunately the three-hour urines were not analyzed for any of the nitrogen fractions.

Until such an experiment is repeated with analyses for all the fractions we can only speculate as to the real cause of the increased excretion of creatin in these particular experiments. If one is prepared to accept the evidence offered by Gottlieb and Stangassinger[43] and by Van Hoogenhuyze and Verploegh[44] that the liver is the chief seat of the transformation of creatin into creatinin, then a higher output of creatin would naturally suggest a diminished power of dehydration in this organ, as Van Hoogenhuyze and ten Doeschate have asserted (see page 424). But one would expect on this hypothesis to witness a corresponding decrease simultaneously, in the amount of creatinin excreted. In two of my experiments (I and IV) there is a slight decrease in the absolute amount of creatinin nitrogen accompanying the increase in creatin nitrogen, but the former is not sufficient to compensate for the latter, and in the other two experiments which reached the later weeks of pregnancy (II and III) there is *an absolute increase in creatinin nitrogen* along with the increase in creatin nitrogen. Van Hoogenhuyze and ten Doeschate have the same phenomenon in three of their normal cases, and in order to save their favorite explanation are obliged to adopt a very cumbrous device. They quote observations by Van Hoogenhuyze and Pekelharing[45] to the effect that creatin is set free by the tonicity of muscle (not by its contraction) and believe that the tonicity of the uterus in the last days of pregnancy is sufficient to account for an increased production of creatin, *a part of which is converted by the liver* (which refuses to convert the normal amount of creatin) *to creatinin!* How much simpler it would be to suppose that the extra creatin and the extra creatinin both originate in the uterus, the former as a product of tonicity perhaps, or as a product of premature involutionary changes, and the latter as a coincidence of increased muscular mass, and that both, being carried to the general circulation, escape by way of the kidney without suffering any change in the liver or elsewhere.

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[42] This journal, 1910, xxvi, p. 134.

[43] Gottlieb and Stangassinger: Zeitschrift für physiologische Chemie, 1907, lii, p. 1, and 1908, lv, p. 322.

[44] Van Hoogenhuyze and Verploegh: Zeitschrift für physiologische Chemie, 1908, lvii, p. 161.

HAS INANITION ANYTHING TO DO WITH THE CHANGED PARTITION IN THE LATE STAGES OF NORMAL PREGNANCY?

The bearing of a protein deficiency on the partition of nitrogen and sulphur is perfectly clear. From the work of Folin[46] we know that a reduction in the nitrogen of the food results in a lower percentage of urea nitrogen and inorganic sulphate sulphur with a corresponding rise in the percentage of uric acid, creatinin, and the undetermined nitrogen, and from the work of Cathcart[47] and of Hawk and his pupils[48] we know that the same is true, though not quite to the same degree, in absolute starvation. Underhill[49] has pointed out that the loss of food by vomiting in the pernicious vomiting of pregnancy must have a profound influence in the same direction. Underhill believes the mere loss of food is sufficient to account for all the departures from normal reported in Ewing and Wolf's cases. This seems to me altogether unlikely. In the first place Underhill has rather underestimated these departures. He finds "a difference of only a few hundredths of a gram of undetermined nitrogen or a matter of 4 or 5 per cent" between the figures of Ewing and Wolf in their cases of "toxemia characterized chiefly by vomiting" and some of those of Folin for "approximately the same output of urine." This is certainly an extreme statement. I have gone over both sets of figures very carefully and find many instances where the percentage of undetermined nitrogen is 7 or 8 per cent higher in the pregnancy cases. Furthermore Underhill fails to note that the same quantity of total nitrogen in the urine does not really represent a similar total metabolism of protein in pregnancy as in a non-pregnant person. As pointed out on page 426, 7.36 gm. nitrogen in the urine of a pregnant woman may mean that 4 or 5 gm., most of it potentially urea nitrogen, have been retained by the product of conception. In pernicious vomiting, where practically all of the food is lost, there is then in the maternal circulation a protein deficiency of a two-fold kind, one due to the quotum taken by the fetus and the other due to failure of the alimentary supply. Under these circumstances it is impossible to believe that the inanition is the sole cause of the urinary changes, especially if the vomiting occur near term. Among Ewing and Wolf's cases the lowest percentage of urea and the highest percentage of undetermined nitrogen occurred in the patient (case 11), who was seized with the attacks of pernicious vomiting late in the pregnancy and who later gave birth to twins. However, the discovery of lactic acid in the urine of pernicious vomiting by Underhill,[50] following its discovery in eclampsia by Zweifel,[51] and by Wolf and Dryfuss[52] indicates a pathological metabolism which separates such cases very sharply from the cases we are here concerned with, and I do not attempt to explain them in the same way.

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[45] VAN HOOGENHUYZE and PEKELHARING: Zeitschrift für physiologische Chemie, 1910, lxiv, p. 262.

[46] *Loc. cit.*, p. 66.

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[47] Cathcart: Biochemische Zeitschrift, 1907, vi, p. 109.

[48] Journal of the American Chemical Society, 1911, xxxiii, pp. 215, 568.

[49] Underhill and Rand: Archives of internal medicine, 1910, v, p. 61.

[50] Underhill: Journal of biological chemistry, 1907, ii, p. 485.

A similar comparison of Ewing and Wolf's figures for their normal cases with those of Folin for "normal" urines justifies the assertion of Ewing and Wolf that in the former the undetermined nitrogen is considerably increased (relatively). Thus taking their five cases, Nos. 1, 3, 4, 5, and 6 (for No. 2 the analyses are not complete) and excluding the post-partum days, we find for the fifteen days an average of 7.2 gm. N in the urine, of which 15.7 per cent is undetermined nitrogen (including creatin nitrogen). Taking the five tables (I to V) of Folin's subjects,[53] and excluding the days upon which the total amount of nitrogen in the urine was either below or above the limits found in Ewing and Wolf's five cases, *i. e.*, 3.7 gm. and 9.7 gm., we find the following:

Table.	Subject.	Total N in urine. Average.	Per cent undet'd N including creatin N in urine. Average.
I	Dr. E. V. S	5.6	9.3
II	O. – F.	5.8	8.4
III	Dr. H. B. H.	6.0	8.0
IV	Dr. E. S. A.	6.2	7.4
V	Dr. Aug. H.	5.8	10.8
Average of all		5.9	8.7

With considerably more than this quantity of nitrogen in the urine of the pregnant women, instead of a lower percentage of undetermined nitrogen than here, as we should expect to find according to Folin's law, we found nearly twice as much. There is, we presume, no question of inanition in any of Ewing and Wolf's normal cases. At all events no mention is made of lack of appetite or of vomiting during the time the observations were made. Hence the altered ratio of undetermined nitrogen must be ascribed to something else; and that something, as I believe, is not diminished oxidation, nor diminished deamination, nor diminished dehydration, but mainly, if not solely, the active growth of the fetus, causing a withdrawal of that nitrogen which otherwise would appear as urea and a consequent relative increase in the other fractions.

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[51] Zweifel: Archiv für Gynaekologie, 1905, lxxvi, p. 537.

[52] *Cf.* Ewing and Wolf: American journal of obstetrics, 1907, lv, p. 305.

[53] Folin: *Loc. cit.*, pp. 70, 74, 76, 78, and 80.

There is a possibility, which I cannot enter into fully at this time, that some products of that digestion which takes place in the placenta leak back into the maternal circulation, and, being carried to the kidney, in part at least, from the general circulation instead of being taken directly to the liver as alimentary products are, are excreted as constituents of the undetermined nitrogen, and probably as undeaminized nitrogen. This would cause an absolute and not merely a relative increase of the undetermined nitrogen in the pregnant as compared with the non-pregnant condition. In Table III may be seen an illustration of the effect of proteolysis (in the resolution of the uterus after parturition) on the undetermined nitrogen. In this case the product of conception was very large (eight puppies) and the amount of proteolytic products reaching the urine directly without conversion in the liver or elsewhere was correspondingly large. It may also be seen from Table V how the undetermined nitrogen increases immediately after conception, at which time we know digestive enzymes are at work in the uterus,[54] and from the other tables how the undetermined nitrogen throughout pregnancy (in the same dog and also in Dog C on the same food) is higher than in sexual rest.

We may surmise that so long as the maternal placenta is not complete the maternal body is not fully protected from such products of digestion. They may be absorbed in such quantity as to be the cause of morning sickness (see paper II, p. 202). If a defect (not necessarily a defect visible to the microscope) were to occur in the maternal placenta, or if the placenta for some reason never became impermeable to such products, would not their leakage back into the maternal circulation account for the toxemia of pregnancy and a part of the high undetermined nitrogen?

Total Phosphoric Acid and Inorganic Chlorides. — My results on these two substances, in the urine of the pregnant dog (Experiment I) are entirely in accord with those of Jägerroos,[55] who found a close parallelism between the nitrogen retention and the phosphoric acid retention, but no such parallelism between the nitrogen and chlorides. In Table I it may be seen that from the fifth week to the end of the pregnancy, where the content of phosphoric acid and chlorides in the food was the same, the phosphoric acid in general goes down as the total nitrogen does, while the inorganic chlorides suffer but little change.

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[54] *Cf.* GRAEFENBURG, Zeitschrift für Geburtshülfe und Gynaekologie, 1909, lxv, p. 1.

[55] JÄGERROOS: Archiv für Gynaekologie, 1902, lxvii, p. 517.

POST-PARTUM METABOLISM.

The metabolism after parturition was not followed continuously for more than a few days in any one experiment, but from the fragmentary evidence thus obtained from each it is possible to piece together a fair conception of the course of events so far as they affect the distribution of the nitrogen and sulphur fractions.

In Experiment I the urine was analyzed on July 1st and 2d, the fifth and sixth days after parturition, and again on July 15th, nearly three weeks thereafter. From this pregnancy only one puppy was born, and it may be seen from the table that the involutionary changes going on during these days was not sufficient to alter the distribution of nitrogen and sulphur very materially from the distribution already noted before parturition. In fact, a comparison of the percentages for the different fractions shows about the same figures both for the nitrogen and sulphur on these days as in the fifth week of the pregnancy. It happens also that the nitrogen retention from the food, for the production of milk, on these post-partum days was very similar in amount to that in the fifth week of pregnancy for the growth of the fetus (*cf.* the columns for nitrogen in the food and total nitrogen in the urine; the amount in the feces was very nearly the same), although the nitrogen balance for the entire week was very different (*cf.* next to last column), owing to the heavy loss which accompanies labor itself.

The figures for July 15th must be taken with some caution, for it is probable that some urine was lost. However the loss could not have been great, and the percentages compare very well with those obtained two weeks after lactation had ceased, in Experiment IV.

In Experiment II the output of nitrogen in the urine on the second and third days after parturition is about what it was during the sixth or seventh week of pregnancy. Active involution is only beginning (see below), and the extra elimination due to ingestion of blood, etc.

by the mother dog at labor is passed. The nitrogen in the urine therefore represents about the same retention for production of milk for the five puppies at the stage of lactation as was being retained for their development and protection in the uterus in the sixth or seventh week. We are prepared then to expect about the same distribution of the nitrogen and sulphur, and a comparison of the percentages shows how reliable this expectation is.

Another illustration is found in Experiment IV. In the fifth week after parturition and the second after lactation had ceased the dog was still retaining nitrogen, and the amount per day was about equal to the amount which was being retained in the eighth week of pregnancy. The percentages are very similar.

In Experiment III one might infer that involution had set in very much earlier, judging by the high undetermined nitrogen on the day following parturition. But this might also be due to the effect of the ingestion of blood and adnexa on the previous day (see note below). The much higher percentage of undetermined nitrogen on the fifth day after parturition with this dog than with Dog A in the first experiment, however, must certainly be due to the greater mass of uterine material in process of resolution, eight puppies having been born from this experiment as compared with one from the former experiments. Whatever influence should be attributed to the lower ingestion of nitrogen (protein) in this experiment would be offset to some extent by the fact that the protein of the food (casein) is much more slowly reduced by digestion to the stage of amino acids than that of the beef heart fed in the other experiments (see page 446.)

It would appear that the creatin nitrogen which has its origin in the uterus comes out before the true proteolytic products, derived from the involution of that organ, which are excreted as constituents of the undetermined nitrogen. Thus in Experiment I the mean quantity of creatin for the fifth and sixth days post-partum is but little more than was present in the fifth week of pregnancy (the time at which the uterus begins to enlarge noticeably), while the quantity of undetermined nitrogen is considerably larger. The creatin from the uterus (only one puppy) must have been eliminated during the first four days; for in Experiment II the creatin nitrogen on the second day after parturition (five puppies) is higher than it had been at any time previous to parturition except the last week, and on the third day it reaches the level of the last week. The undetermined nitrogen has not yet been affected. In Experiment III, while both the creatin nitrogen and the undetermined nitrogen reach their highest absolute values on the fifth day (December 8), by the seventh day the creatin nitrogen has fallen much lower (relatively) than the undetermined nitrogen.[56] The creatin in this experiment, it must be remembered, is all of endogenous origin.

SUMMARY AND CONCLUSIONS.

1. The departures from "normal" standards of the nitrogen and sulphur partitions in the urines of normal pregnancy can be explained as the result of protein retention.

2. Low urea nitrogen or low urea and ammonia nitrogen combined is accounted for by the fact that the nitrogen, which under ordinary circumstances would be split off from amino compounds as ammonia and eliminated in the form of ammonia salts or in the form of urea, is resynthesized in the intestinal wall to make good the depletion of the blood proteins caused by the product of conception (fetus, uterine wall, and adnexa).

Those fractions of the urinary nitrogen which indicate the extent of the endogenous metabolism, namely, creatinin, uric acid (in part), and undetermined nitrogen (in part), show as a consequence a relative, increase.

3. The same is true to a large extent of the inorganic sulphate sulphur and the unoxidized or neutral sulphur. The former is held back as a consequence of fetal development, while the latter is relatively, but not absolutely, increased.

4. One dog kept on a creatin-free food for five days previous to parturition showed a small quantity of creatin in the urine on the days immediately preceding parturition. In three other experiments with creatin in the food the quantity of creatin in the urine increased very noticeably during the last week of pregnancy. This is probably indicative of tissue changes in the uterus (tonicity or involution) rather than of deficient power of dehydration in the liver.

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[56] The day of parturition must be excepted because of the large ingestion of blood and adnexa by the mother dog during labor. The high undetermined nitrogen on this day is not due to involution in the proper sense of the term.

5. The distribution of the urinary fractions of nitrogen and sulphur after parturition follows Folin's law of dependence upon the total nitrogen in the urine, or, since the quantity of nitrogen in the food was the same and the amount in the feces nearly the same, it depends upon the amount of nitrogen being retained, just as before parturition. In the involutionary changes the creatin of the uterine substance is set free before the proteolytic products.

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